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EVALUATING FLEECE QUALITY OF NAVAJO SHEEP FROM SMALL SAMPLES[1]

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INTRODUCTION

Navajo sheep produce a mixed type of wool. The fleece has an undercoat of true wool fibers and a protective outercoat of long, coarse, hairlike fibers. In common with other wools of this type, the fleece also contains varying quantities of kemp and other medullated fibers.

Kemp is a distinct type of fiber that differs both in form and manner of growth from other medullated fibers of the undercoat and outercoat and can usually be detected by visual inspection of the fleece. Macroscopically the fibers are typically short, coarse, straight, or waved (uniplanar) with a long, tapering tip and an opaque appearance. When examined microscopically, kemp is found to consist largely of medullae, which are built up of superimposed cells that usually contain air. Duerden (*1*)[2] has shown that the inclusion of air in the medullae is responsible for the paleness or off color of the kemp in dyed wool. Kemp fibers are shed periodically and replaced by new growth, a characteristic that is not associated with other types of medullated fibers found in the fleeces of Navajo sheep.

Medullation is often considered a basis for distinguishing hair from wool, but it is not a positive criterion. In Navajo fleeces the medullated fibers are found principally in the coarse, hairy outercoat, but small numbers of the undercoat or true wool fibers frequently have a fine medullary canal. The medullae correspond to the three types described and illustrated by Wilson (*9*) and Von Bergen and Mauersberger (*8*) as fragmental, interrupted, or continuous, and vary in size from approximately 10 to 60 percent of the cross-sectional area of the fiber.

Outercoat, kemp, and other medullated fibers are the dominant factors that influence the economic and utility values of Navajo wool. Grandstaff (*4*) has shown that the suitability of the wool for hand weaving varies in direct relation to the quantities of kemp and hair fibers present in the fleece. In the tests made by him, rugs woven from Navajo wool containing fewer than 1 percent of kemp fibers and 10 percent of outercoat fibers greater than 40 microns in diameter were of the highest quality. Kemp in amounts of 3 to 5 percent interfered with the spinning of uniform yarn and resulted in rugs of

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[2] Italic numbers in parentheses refer to Literature Cited, p. 191.

coarse, harsh texture. Rugs made from wool containing 15 to 25 percent of outercoat fibers were of coarse, hairy texture and inferior quality.

The quantities of outercoat, kemp, and other medullated fibers vary considerably between regions of individual fleeces, and to a much greater extent between the fleeces of different sheep produced under similar environmental conditions. Hence, these variations are largely if not wholly due to the genetic composition of the sheep.

Progress in fleece improvement, through selective breeding of the sheep, depends on a reliable system of fleece sampling and analysis. The objectives of this study were to measure the distribution of outercoat, kemp, and other medullated fibers in the fleeces; to determine the minimum size of samples required for laboratory analysis; and to select certain definite anatomical areas which, individually or combined, provide the best index of fleece quality.

MATERIALS AND METHODS

At weaning time in the fall of 1939, 24 Navajo ewe and ram lambs were selected at random for a fleece-sampling test at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex. Preparatory to sampling, each lamb was placed on a table, and the neck, shoulder, side, and thigh regions were blocked out on the left half of the body with marking chalk. Three lines were so drawn as to divide these 4 fleece regions equally. The belly region was also divided into 4 quarters. Samples of wool about the size of 3 fingers were taken with an electric clipper from the 44 positions indicated in figure 1,

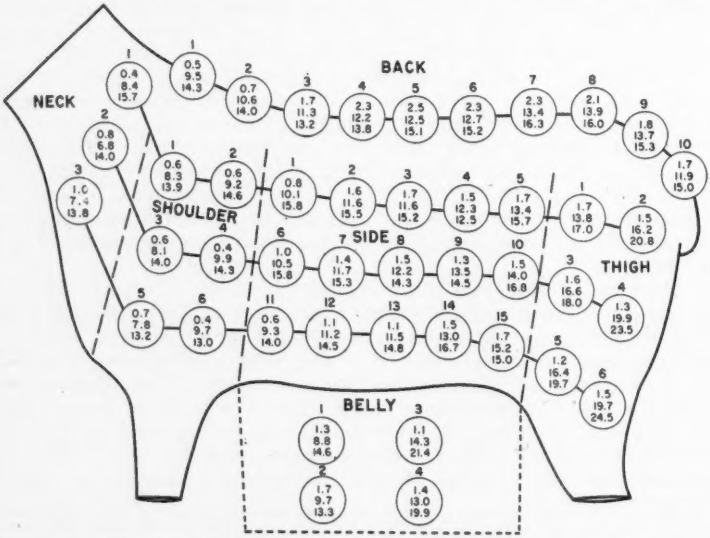

FIGURE 1. The location of positions sampled in each of the 6 regions of the fleece is indicated by numbered circles. Data from top to bottom within the circles represent the average numbers of kemp, other medullated fibers, and outercoat fibers, respectively, per 100 total fibers, observed for each position.

thus making a total of 1,056 samples for the 24 lambs. Distribution of the 44 sample positions was as follows: 10 were located at regular intervals along the backbone from the point of the withers to the tailhead, 10 along each of the 3 lines that were used to divide the left half of the body, and 1 at approximately the center of each of the 4 quarters of the belly. The total number of positions for the back was 10; neck, 3; shoulder, 6; side, 15; thigh, 6; and belly, 4.

In the laboratory each fleece sample was first divided into 11 subsamples of the same size, 10 of which were used for counts of kemp and other medullated fibers, and the eleventh for measurements of the fiber-diameter distribution. A tuft of fibers from each subsample was cross-sectioned by means of Hardy's (*6*) device and projected on a ground-glass field 7 by 11 inches in size. From the magnified image of the fiber cross sections, a representative portion of 100 fibers was used for counting the number of kemp and other medullated fibers in subsamples 1 to 10 and for making the diameter measurements of subsample 11. The fibers were measured by the method reported by Grandstaff and Hodde (*5*), and the total number of fibers more than 40 microns in diameter, exclusive of kemp, was used as the best estimate of the amount of outercoat in each subsample.

Before analyzing the data for the three types of fibers, all observations were increased by 1 for the purpose of eliminating zeros occasioned by the absence of kemp and other medullated fibers in some samples. The data were studied by analysis of variance, according to the method of Snedecor (*7*), and the adjustments of Ezekiel (*2*) for small size of samples were applied to all correlation and regression coefficients.

EXPERIMENTAL RESULTS

DISTRIBUTION OF FIBERS

Figure 1 shows the average distribution of the 3 types of fibers in the 44 positions of the fleece, based on the mean number of fibers per subsample. Variations in the number of outercoat and medullated fibers other than kemp were closely associated, whereas the amount of kemp varied independently of the other 2 types. In general, all 3 types increased pronouncedly from the neck or shoulder to the thigh. The back, however, exceeded all other regions of the fleece in quantity of kemp. The ranges in mean numbers of kemp, other medullated fibers, and outercoat fibers for the 6 regions were as follows: 0.5 to 1.8, 7.5 to 17.1, and 13.8 to 20.6, respectively. The side approximated the average of the 44 positions for the various types of fibers considered. Within fleece regions the amounts of kemp, other medullated fibers, and outercoat fibers did not vary in a systematic manner.

In order to evaluate the differences in number of the three types of fibers, the data for each type were studied separately by an analysis of variance. Analyses for kemp and other medullated fibers were based on three criteria of classification for each of the six body regions: Between sheep, between positions, and between subsamples.

Within each region, differences between sheep constituted the primary source of variation for kemp. Except for the belly region, this statement also applies to other medullated fibers. In all cases the F values were many times as great as the value required at the 1-percent level of significance. For kemp the variance due to differences

between positions was significant for the neck and highly significant for the back and side. For medullated fibers other than kemp, differences between positions represented a highly significant source of variance in all 6 regions of the fleece. The variance due to differences between the numbers of kemp fibers in 10 subsamples for each position was not significant in any fleece region, whereas for the other medullated fibers the variance between subsamples was highly significant in all regions of the fleece.

The variance due to interaction of sheep × position was considerably larger in most instances than the variance due to differences between subsamples. For kemp the *F* values for sheep × position were not significant except for the back and belly, whereas for medullated fibers they were highly significant for all regions of the fleece. The variances due to interactions of sheep × samples and positions × samples were also substantially greater for other medullated fibers than for kemp.

Separate analyses of the total numbers of kemp and other medullated fibers for the six fleece regions showed that the differences between regions were highly significant.

In the data on outercoat fibers differences between sheep were also a major source of variation. An analysis of the variance within and between positions and regions of the fleece showed that differences between positions were significant for the thigh and belly only, and differences between regions of the fleece were highly significant.

MINIMUM SIZE OF SAMPLES REQUIRED

From a practical standpoint the size and number of samples required for evaluating quality of whole fleeces are factors of primary importance. The usual practice at the Southwestern Range and Sheep Breeding Laboratory has been to sample the fleece of each sheep from the middle of the side, thigh, and back, and to use a sample of 100 fibers from each of the 3 positions for evaluating average amounts of outercoat, kemp, and other medullated fibers.

To determine the minimum size of sample required for measuring the amounts of kemp and other medullated fibers within fleece positions, data for the 10 subsamples of position 1 in each fleece region were studied by analysis of variance. For this purpose the data were grouped so that the variance of the first subsample was compared with that of the first 3, first 5, and all 10 subsamples for each of the respective positions. It was found that the variance within samples of 100, 300, 500, and 1,000 fibers was much greater than the variance between the 4 different-sized samples for each position. It appeared, however, that the results of this analysis might have been somewhat biased, since subsample 1 was also a part of the 3, 5, and 10 subsamples with which it was compared. To eliminate the possibility of bias, the variance of subsample 1 was compared with that of the next 3 (subsamples 2 to 4, inclusive), next 5 (subsamples 2 to 6), and last 9 (subsamples 2 to 10). Here again the variance within the different-sized samples was found to be significantly greater than the variance between samples. These results indicated that a sample of 100 fibers was as reliable as a sample of 1,000 fibers for detecting real differences between sheep in number of kemp and other medullated

fibers in each fleece position studied. On the basis of these facts, it appeared safe to assume that a sample of 100 fibers would also provide a reliable estimate of the amount of outercoat in each fleece position. It was realized, however, that a sample of more than 100 fibers might be required for evaluating differences between sheep when the fleeces are more uniform than those in the present study.

AREAS PROVIDING BEST INDEX OF FLEECE QUALITY

As a basis for selecting a representative position within each fleece region, the numbers of kemp and other medullated fibers in the first subsample from each position were studied by analysis of variance. The results showed that in all regions except the belly, the variance within positions was significantly greater than the variance between positions, when the 24 sheep were considered as a unit. Consequently, it appeared that the neck, shoulder, side, thigh, and back could each be evaluated from a single sample of 100 fibers taken from any one of the several positions sampled within each of these regions. The following positions were therefore chosen arbitrarily as representative of their respective regions: Neck, 2; shoulder, 3; side, 7; thigh, 3; back, 5 (fig. 1). The positions indicated for the side, thigh, and back are similar to those ordinarily used when sampling the fleeces for the 3 types of fibers. Since the belly represented a small and relatively unimportant part of the fleece, position 1 was arbitrarily chosen for this region even though the analysis of variance showed that there were significant differences between the belly positions.

With the use of 6 selected positions, the data were analyzed by means of simple and multiple correlations and regressions. These positions were correlated with one another and with the remaining 38 positions for numbers of the 3 types of fibers. In this study the 38 positions represented the best measure of the whole fleece. From a comparison of the interposition correlations for each type of fiber it was impossible to show any consistent relationship between the fiber content of adjacent and more distant parts of the fleece. In the kemp fibers, for instance, the contiguous regions, neck-shoulder, shoulder, side, side-thigh, and neck-thigh, had correlations of +0.79, +0.46, +0.48, and +0.63, respectively. When all the correlations were considered, however, 11 of those for kemp, 10 for other medullated fibers, and 12 for outercoat fibers were significant or highly significant. The correlations between the belly sample and the other 5 positions tended to be low and statistically insignificant, especially for outercoat fibers and medullated fibers except kemp. On the other hand, the correlations for the side and thigh with each of the remaining 4 selected positions exceeded the expected P value of 0.404 required at the 5-percent level of significance and were of about the same magnitude for outercoat, kemp, and other medullated fibers.

Table 1 shows that the neck, shoulder, side, thigh, and back have highly significant positive correlations with the other 38 positions for the 3 types of fibers. The correlation coefficients for these 5 positions ranged from +0.66 to +0.88 for kemp, +0.62 to +0.89 for other medullated fibers, and +0.72 to +0.88 for outercoat fibers. Differences between the correlation coefficients for each of the 3 kinds of

fibers were tested by means of Fisher's (3) z transformation and found to be statistically insignificant. The correlation for the thigh was highest for kemp, whereas that for the side was highest for other medullated fibers and outercoat fibers. The correlations for the belly and the 38 unselected positions were consistently low, but they were significant in all cases.

TABLE 1.—*Relation between average numbers of the 3 types of fibers in samples from 38 fleece positions (Y) and 1 selected position (X) from each of the 6 regions*

Fleece region and position [1]	Adjusted correlation coefficients [2]	Regression coefficients [3]		Standard errors of estimate	Reduction from standard deviation of 38 positions to standard error of estimate
		a	$by.x$		
KEMP FIBERS					
					Percent
Neck, 2	0.67	0.92	0.94	1.45	26
Shoulder, 3	.66	.65	1.21	1.48	25
Side, 7	.75	.91	.72	1.30	34
Thigh, 3	.88	.81	.60	.94	52
Back, 5	.76	.69	.50	1.27	36
Belly, 1	.50	1.34	.53	1.70	13
OTHER MEDULLATED FIBERS					
Neck, 2	0.62	7.88	0.73	4.65	21
Shoulder, 3	.78	6.42	.76	3.70	37
Side, 7	.89	3.20	.76	2.63	55
Thigh, 3	.78	4.37	.52	3.70	37
Back, 5	.75	2.53	.84	3.94	33
Belly, 1	.49	7.50	.55	5.15	13
OUTERCOAT FIBERS					
Neck, 2	0.79	7.52	0.60	3.78	38
Shoulder, 3	.74	8.57	.52	4.09	33
Side, 7	.88	6.08	.64	2.87	53
Thigh, 3	.72	6.23	.53	4.24	31
Back, 5	.73	4.68	.74	4.20	31
Belly, 1	.51	10.72	.35	5.26	14

[1] Location of each position shown in figure 1.

[2] For 22 degrees of freedom the 5-percent level of significance is 0.404, and the 1-percent level of significance is 0.515. All correlation coefficients are positive.

[3] a and b are the coefficients necessary to the expression of any linear regression. All regression coefficients are positive.

The regression coefficients, $by.x$, were used to establish the predictive value of each of the 6 selected positions in estimating the average fiber content of the 38 positions. The information obtained by solving the regression equations is also given in table 1. The thigh sample had the lowest standard error of estimate for kemp fibers and accounted for 52 percent of the variance found in the 38 positions. For the other medullated fibers and the outercoat fibers the side sample had the lowest standard error of estimate and reduced the variance of the 38 positions by 55 and 53 percent, respectively.

From the data presented in table 1, it is apparent that some combination of two or more positions should be used in evaluating the fleece. The 15 possible pairs of selected positions were correlated with the remaining 38 positions in order to determine which combination would

yield the most information. All multiple correlation coefficients for the 3 types of fibers exceeded the P value of 0.596 required at the 1-percent level of significance. The coefficients ranged from +0.68 to +0.95 for kemp, +0.72 to +0.95 for other medullated fibers, and +0.79 to +0.93 for outercoat fibers. The combination of the side and thigh yielded the highest correlation for kemp and other medullated fibers (table 2) and next to the highest for outercoat fibers. Moreover, the coefficients of multiple determination showed that 77 to 90 percent of the variation in numbers of kemp fibers in the 38 positions could be accounted for when the thigh sample was one of the two positions considered. In the other medullated fibers and the outercoat fibers, the use of the side sample as one of the two positions accounted for 70 to 91 percent, and 79 to 87 percent, respectively, of the variance of these types of fibers in the 38 positions.

TABLE 2.—*Relation between the average numbers of the 3 types of fibers in samples from 38 fleece positions (Y) and 1 selected position from each of the side and thigh* [1]

Type of fiber	Adjusted multiple correlation coefficients [2]	Net regression coefficients [3]			Standard error of estimate	Reduction from standard deviation of 38 positions to standard error of estimate
		a	$by.x_1$	$by.x_2$		
						Percent
Kemp	0.95	0.32	0.40	0.45	0.63	68
Other medullated	.95	1.52	.56	.24	1.83	72
Outercoat	.92	3.76	.50	.25	2.38	61

[1] Selected positions were side 7 (X_1) and thigh 3 (X_2).
[2] For 21 degrees of freedom the 1-percent level of significance is 0.596. All correlation coefficients are positive.
[3] a and b are the coefficients necessary to the expression of any linear regression. All regression coefficients are positive.

The combination of side and thigh yielded the best estimate of the 38 positions, when all 3 fiber types were considered in evaluating the fleece. For kemp this combination accounted for 68 percent, for other medullated fibers 72 percent, and for outercoat fibers 61 percent, of the variance found in the 38 positions. In the outercoat fibers, the neck and side accounted for 3 percent more of the total variance of the 38 positions than the side and thigh. This small gain for outercoat fibers did not seem to justify the use of the neck sample, since it proved to be less reliable than the thigh as an index of the amounts of kemp and other medullated fibers in the 38 positions.

The net regression coefficients for the side and thigh combination were positive for all 3 types of fibers studied. This means that for every unit increase in fiber content of the side and thigh, the amount of change in the 38 positions would be the sum of the increases for the 2 positions. Within the limits of this study, however, the coefficients of determination showed that for kemp 23 percent, for other medullated fibers 31 percent, and for outercoat fibers 29 percent, of the variance were common to both the side and thigh. The actual increase in fiber content of the 38 positions, therefore, would be less

in some instances than that determined mathematically from the multiple regression equations.

Since the fleeces have been sampled heretofore from the side, thigh, and back, it was considered advisable to compare these 3 positions, as well as the 6 positions representing all regions of the fleece, with the average of the 38 positions. The multiple correlations obtained for these 2 sets of comparisons were as follows: Kemp, +0.95 and +0.95; other medullated fibers, +0.97 and +0.97; and outercoat fibers, +0.93 and +0.95, respectively. Comparison of these correlation coefficients with those obtained for 2 positions (side and thigh) and the 38 positions showed no differences for kemp and only slight increases for other medullated fibers and outercoat fibers.

The use of the side, thigh, and back accounted for only 2 percent more of the total variance for outercoat fibers and 3 percent for medullated fibers other than kemp, than the use of the side and thigh. The use of the 6 positions reduced the total variance for outercoat and medullated fibers other than kemp by 5 percent in each case, but only 1 percent for kemp, as compared with the use of only the side and thigh. Thus it appears that little is gained by using more than the side and thigh as a basis for estimating the amounts of outercoat, kemp, and other medullated fibers in the fleece.

APPLICATION OF RESULTS

In applying the prediction equations for each of the 3 variables (kemp, other medullated fibers, and outercoat fibers), the average for 38 positions (Y) may be estimated from the values obtained for the side (X_1) and thigh (X_2) by the use of the formula $\overline{Y}=a+by{\cdot}x_1X_1+by{\cdot}x_2X_2$. The a and b values given in table 2 for the 38 positions, position 7 of the side, and position 3 of the thigh are applied in solving this equation. Thus the predicted number of kemp fibers for the 38 positions, from observed values of 2 kemp for the side and 6 kemp for the thigh, would be calculated as follows: $0.32+(0.40\times2)+(0.45\times6)=3.82\pm0.63$.

In applying the prediction equation to single fleeces, it should be remembered that the standard errors of estimate define the range within which approximately two-thirds of the predicted $\overline{Y}$ values will fall. Consequently, the estimated totals of the three types of fibers for about one-third of the fleeces may be either larger or smaller than their true values. Nevertheless, the limits of accuracy inherent in the method of fleece sampling and evaluation presented herein are considered to be well within the bounds of control that can be accomplished in actual breeding practice with Navajo sheep.

The predicted fleece values for the three types of fibers at weaning age will be useful in conducting progency tests of the rams used each year. Errors made in evaluating individual fleeces will tend to compensate each other when the data are combined and analyzed for progeny groups. Consequently, it should be possible to detect fleece differences between progeny groups with a high degree of certainty.

Although the purpose of this study is to illustrate a method of evaluating the fleeces of Navajo sheep from small samples, it should also be useful in conducting fleece-sampling tests with other types of sheep.

SUMMARY

A fleece-sampling test was conducted at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex., to develop a practical and reliable system of evaluating the amounts of outercoat, kemp, and other medullated fibers in the fleeces of Navajo sheep at weaning age. The work was conducted in the fall of 1939 with 24 ewe and ram lambs. Wool samples were taken from 44 positions on the back, belly, neck, shoulder, side, and thigh of the left half of the body of each animal.

Throughout the fleece, variations in the amount of outercoat fibers and medullated fibers other than kemp were closely associated, whereas kemp varied independently of the other two types.

By means of analyses of variance the following significant facts were established: (1) Within fleece regions the differences between sheep were the major source of variance in all but one instance; (2) the variance between fleece regions was many times as large as the variance within regions; (3) when the 24 sheep were considered as a unit, a sample of 100 fibers proved to be as reliable as 1,000 fibers for detecting significant differences between sheep; (4) the variance within positions, based on samples of 100 fibers, was significantly greater than the variance between positions, except for the belly region.

Comparison of 1 selected position for each of the 6 fleece regions with the average of the remaining 38 positions yielded significant or highly significant correlations, the thigh being highest for kemp and the side highest for other medullated fibers and outercoat fibers.

For evaluating the fleeces, combinations of 2, 3, and 6 positions were compared with the average of 38 positions. All multiple correlation coefficients were highly significant. Of the various combinations of 2 positions, the side and thigh ranked highest for kemp and other medullated fibers and next to the highest for outercoat fibers. These two positions reduced the total variance 68, 72, and 61 percent, respectively, for the 3 types of fibers. Combinations of 3 and of 6 positions proved to be slightly superior to the combination of side and thigh for evaluating outercoat fibers and medullated fibers other than kemp. From a practical standpoint, however, the results did not justify the use of more than these 2 positions, particularly when the 3 types of fibers were considered.

The 3 regression equations for 38 positions on the side and thigh provide the necessary information for use in estimating the average amounts of the 3 types of fibers in the fleece from data for the 2 selected positions.

LITERATURE CITED

(1) DUERDEN, J. E.
1926. KEMP FIBRES IN THE MERINO. Textile Inst. Jour. 17: T268–T273, illus.

(2) EZEKIEL, M.
1930. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London.

(3) FISHER, R. A.
1941. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 8, rev. and enl., 344 pp., illus. Edinburgh and London.

(4) GRANDSTAFF, J. O.
1942. WOOL CHARACTERISTICS IN RELATION TO NAVAJO WEAVING. U. S. Dept. Agr. Tech. Bul. 790, 36 pp., illus.

662980—45——2

(5) GRANDSTAFF, J. O. and HODDE, W. L.
1940. A RAPID METHOD FOR PROJECTING AND MEASURING CROSS SECTIONS OF WOOL FIBERS. U. S. Dept. Agr. Cir. 590, 11 pp., illus.
(6) HARDY, J. I.
1933. DETERMINATION OF FIBRE FINENESS. A RAPID METHOD USING A NEW CROSS SECTIONING DEVICE. Textile Res. 3: 381–387, illus.
(7) SNEDECOR, G. W.
1940. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed. 3, 422 pp., illus. Ames, Iowa.
(8) VON BERGEN, W., and MAUERSBERGER, H. R.
1938. AMERICAN WOOL HANDBOOK . . . 864 pp., illus. New York.
(9) WILSON, J. F.
1929. THE MEDULLATED WOOL FIBER. Hilgardia 4: [135]–152, illus.

MYCOSPHAERELLA BLACK ROT OF CUCURBITS[1]

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INTRODUCTION

New York, N. Y., importers of cucumbers (*Cucumis sativus* L.) have in recent years suffered heavy losses from decay in boat shipments from Puerto Rico and Cuba.[2] Mycosphaerella black rot, caused by *Mycosphaerella citrullina* (C. O. Sm.) Gross. (*12*),[3] was found to account for most of the spoilage. Investigation of this disease was therefore begun at the Market Pathology Laboratory of the United States Department of Agriculture at New York in 1938 and was continued at irregular intervals through 1942. The study also included observations on the decay of other cucurbits of both foreign and domestic origin.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Many investigators have noted the occurrence of cucurbit diseases caused by *Mycosphaerella citrullina*. Symptoms may include spotting of the leaves; production of cankers on stems, petioles, and fruitstalks; decay of the stem; wilt of the vine; and rotting of the fruit. A summary of many such reports is given in table 1.

On the market Orton and Meier (*22*) found *M. citrullina* causing both stem-end and blossom-end rots of Florida and Georgia watermelons at New York. Market observations of a mycosphaerella black rot of cucumber fruits were first made at New York by F. C. Meier.[4] These were followed by more complete transit and market studies by Meier, Drechsler, and Eddy (*18*).

Brief reports based on the studies here summarized have already appeared (*4, p. 47*).[5] [6]

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[1] Received for publication September 9, 1943.

[2] Processed reports issued by the Agricultural Marketing Service, U. S. Department of Agriculture, show that from 1938 to 1941, inclusive, an average of 3,061 carlots of cucumbers were unloaded annually at New York, N. Y., of which 226 carlots (7.4 percent) were received from Puerto Rico and Cuba (Isle of Pines). Most of these imports arrived during the 4 months of December to March and constituted 80.7 percent of all cucumbers received annually at New York during that period.

[3] Italic numbers in parentheses refer to Literature Cited, p. 211.

[4] HASWELL, R. J., and WOOD, J. I. DISEASES OF FIELD AND VEGETABLE CROPS IN THE UNITED STATES IN 1920. U. S. Bur. Plant Indus., Plant Dis. Bul. Sup. 16, pp. 177–287. 1921. [Processed.] (See pp. 251 and 252.)

[5] BRATLEY, C. O., and WIANT, J. S. DISEASES OF FRUITS AND VEGETABLES ON THE NEW YORK MARKET . . . U. S. Bur. Plant Indus., Plant Dis. Rptr. 23: 161. 1939. [Processed.]

[6] WIANT, J. S., and BRATLEY, C. O. DISEASES OF FRUITS AND VEGETABLES ON THE NEW YORK MARKET . . . U. S. Bur. Plant Indus., Plant Dis. Rptr. 24: 363; 25: 332. 1940–41. [Processed.]

TABLE 1.—*Cucurbit diseases caused by Mycosphaerella citrullina*

Suscept	Disease reported	Location	Source of report
Cucumis sativus (cucumber).	Mycosphaerella wilt or gummy stem blight of vines; black rot of fruits.	Florida, Massachusetts, New Jersey, New York, and Trinidad, British West Indies.	Weber (*36*); Boyd (*5, p. 207*); Wardlaw, Leonard, and Baker (*35*).[1] [3]
Cucumis melo L. (muskmelon).	Stem canker, mycosphaerella wilt, or gummy stem blight of vines; black rot of fruits.	Florida, Massachusetts, New York, and Japan.	Hemmi (*15*); Sherbakoff (*30*); Boyd (*5, p. 207*).[2] [3] [4]
Citrullus vulgaris Schrad. (watermelon).	Mycosphaerella wilt or gummy stem blight of vines; decay of fruits.	Alabama, Arizona, Florida, Georgia, Louisiana, Massachusetts, Mississippi, Missouri, New York, South Carolina, Virginia, and Puerto Rico.	Sherbakoff (*30, 31*); Walker (*33, p. 88*); Walker and Weber (*34*); Orton (*21*); Orton and Meier (*22*).[2] [5]
Cucurbita maxima Duchesne (winter squash).	Gummy stem blight of vines; black rot of fruits.	Maine, Massachusetts, and Michigan.	Kauffman (*14*); Boyd (*5, p. 207*).[2] [6]
Cucurbita pepo L. (summer squash, pumpkin, vegetable marrow and warty gourd).	Wilt of vines; black rot of fruits.	Massachusetts, Michigan, New Jersey, New York, and Union of Soviet Socialist Republics.	Boyd (*5, p. 207*); Rodighin (*26*).[2] [7]
Cucurbita moschata Duchesne (Cushaw squash).	Vine wilt; fruit rot	Massachusetts	Boyd (*5, p. 207*).
Sechium edule (Jacq.) Swartz (chayote).	Not specified	Florida	(1).

[1] See also pp. 251 and 252 of reference in footnote 4, p. 193.
[2] Also various numbers of Plant Disease Reporter and Plant Disease Reporter Supplement issued by the Bureau of Plant Industry. [Processed.]
[3] CHUPP, C. MYCOSPHAERELLA WILT OF MELONS IN NEW YORK. U. S. Bur. Plant Indus., Plant Dis. Rptr. 16: 143–144, 1932. [Processed.]
[4] In a letter of October 4, 1938, to the author, Dr. Charles Chupp reported again finding muskmelon fruits affected with an advanced stage of mycosphaerella black rot.
[5] Numerous annual reports of Florida Agricultural Experiment Station.
[6] Numerous annual reports of the Massachusetts Agricultural Experiment Station.
[7] See also footnote 8, p. 207.

DECAY OF CUCUMBERS ON THE MARKET

Decay was prevalent in many Puerto Rican shipments of cucumbers received during each of the four winter seasons 1938–39 to 1941–42, inclusive. Mycosphaerella black rot was regularly the most important type of decay found. In many shipments it alone was responsible for all the spoilage noted.

Losses from black rot were extremely variable in both distribution and amount. In many cargoes practically none occurred. In others the crates of many brands had from a few to practically all of their contents affected with this decay.

The representative of one large group of shippers has estimated that damage from decay was reflected to an important extent in the lower sale price received for approximately 31 percent of 20,000 crates imported during the 1939–40 season. Most of the decay was mycosphaerella black rot. This decay was likewise the most important one found by Federal food-products inspectors during their examination of Puerto Rican lots totaling over 23,000 crates (table 2), in which approximately 23 percent of the fruits were spoiled.

Imports from Cuba (table 2) were found affected with black rot throughout the 4-year period, although losses were not so great as those in imports from Puerto Rico.

Only once was mycosphaerella black rot observed on domestic cucumbers; the diseased fruits were found in a rail shipment from Georgia that arrived at New York during the summer of 1938.

TABLE 2.—*Occurrence of mycosphaerella black rot on cucumbers at the New York market* [1]

Origin	Season	Inspections made	Crates inspected	Fruits decayed [2]
		Number	*Number*	*Percent*
Puerto Rico	1938–39	16	2,920	11.0
	1939–40	11	2,459	6.2
	1940–41	21	9,336	10.4
	1941–42	29	8,783	38.9
Cuba [3]	1939–40	6	1,584	8.5
	1941–42	2	486	9.6

[1] Data based on observations made by inspectors of the Agricultural Marketing Administration, U. S. Department of Agriculture.
[2] Mostly mycosphaerella black rot.
[3] Black rot was observed in 21 other shipments during the 4 seasons. Observations made (at least in part) on fruit collected by inspectors of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.

DECAY OF OTHER CUCURBITS ON THE MARKET

Though of commercial importance only on Puerto Rican and Cuban cucumbers, mycosphaerella black rot was noted on several other domestic and imported cucurbits. The results of these observations are summarized in table 3.

Only limited data were obtained on watermelon from the Southern States or on domestic squash. Consequently it cannot be stated definitely to what extent black rot is found on these cucurbits.

Extensive observations on cantaloups and on Honey Dew, Honey Ball, and related melons have been made continuously at this market for a number of years. In no instance has mycosphaerella black rot been found on any of these domestic muskmelons. Nor has it ever been found during the course of frequent observations of cantaloups imported from Mexico, or on Honey Dew melons from Chile and Argentina. The occurrence of black rot on the muskmelon fruit presumably originating in Venezuela (table 3) is thus of unusual interest.

TABLE 3.—*Occurrence of mycosphaerella black rot on cucurbits other than cucumbers at the New York market*

Suscept	Origin	Notes
Watermelon	Southern States	Few observations made; black rot not found.
	Cuba	Black rot found affecting a few melons in shipment received in mid-September 1940.
Hubbard squash (*Cucurbita maxima*).	Massachusetts	Noted on several fruits sent from Boston, Mass., market by Agricultural Marketing Administration inspector.
	Virginia	Carlot with 20 percent of the fruit affected by black rot, mid-December 1940.
Yellow Crookneck squash (*Cucurbita pepo*).	Cuba	*Mycosphaerella citrullina* found fruiting on stem attachments of several fruits, February 1940.
Chayote	Jamaica, British West Indies; Cuba.	Black rot of fruits observed on numerous occasions during January and February 1940 mostly scattered in small lots.[1]
	Puerto Rico	Black rot of fruits noted on several occasions.
Balsam pear (*Momordica charantia* L.).	Cuba	Black rot of fruits noted on 5 occasions during January and February 1940.[1]
Chinese preserving melon (*Benincasa hispida* (Thunb.) Cogn.).	do	Black rot of fruit noted January 1937.[1]
Muskmelon	Venezuela (?)	Single fruit with black rot found in ship's stores of a vessel arriving from Venezuela.[1]

[1] Observations made (at least in part) on fruit collected by inspectors of the Bureau of Entomology and Plant Quarantine.

SYMPTOMS

The first symptoms of the disease on cucumber fruits are small, dark, greasy or water-soaked spots (fig. 1, *A* and *B*), which may occur anywhere on the surface of the fruits. These spots may be roughly

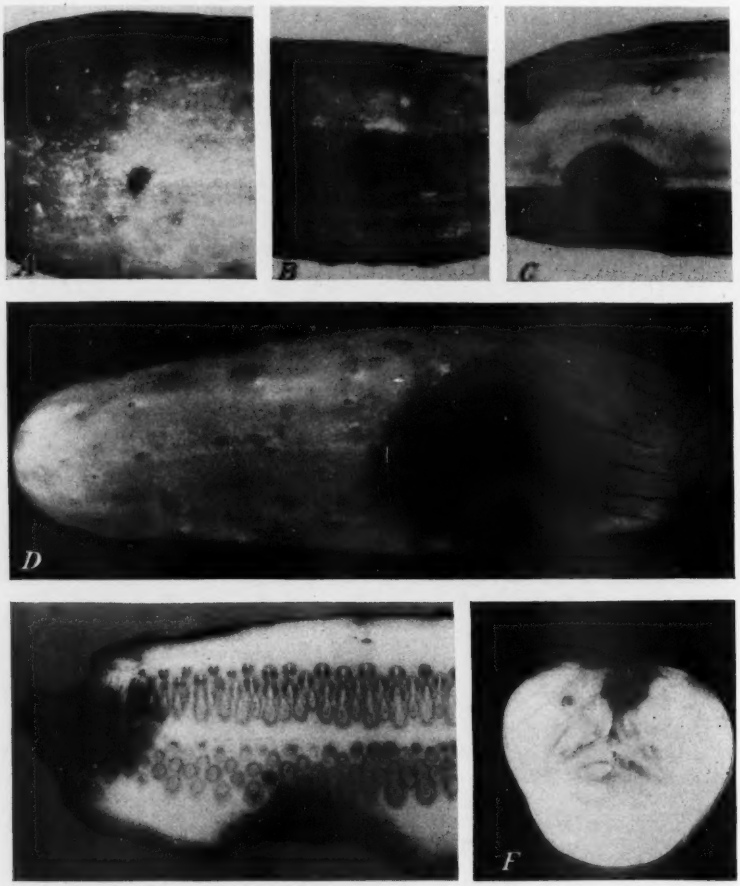

FIGURE 1.—Mycosphaerella black rot of cucumber: *A–C*, Early stages, with gummy exudate shown in *C*; *D*, advanced stage, showing shriveling, wrinkling, and blackening; *E*, longitudinal section through lesion; *F*, cross section through lesion.

circular with well-defined margins, or they may be of irregular shape with indefinite margins. Frequently a gummy exudate develops at the center of the lesion and dries, forming a firm deposit (fig. 1, *C*). This, however, is not an invariable symptom.

On dark-green cucumbers that may be described as "grass green" [7] or "spinach green" and on lighter green fruits that are "parrot green"

[7] Color designations in quotation marks are according to Ridgway (*25*).

and "courge green" to "Biscay green," the lesions in early stages are variously "yellowish olive," "dark greenish olive," "deep olive," or "Lincoln green." The more advanced lesions on lighter colored or more yellowed fruits are frequently "honey yellow," "Isabella color," or "buffy citrine."

During the early stages, particularly if the color changes are not marked and the gummy exudate is prominent, the decay might be mistaken for bacterial spot, caused by *Pseudomonas lachrymans* (E. F. Sm. and Bryan) Ferraris. Larger, darker spots in which no exudate has formed may, on the other hand, resemble early lesions of bacterial soft rot, caused by *Erwinia carotovora* (Jones) Holland, in which extensive softening has not yet occurred.

Although lesions may become considerably advanced without turning dark, the most important symptom of the later stages of decay is the blackish discoloring of the affected areas (fig. 1, *D*) that first develops at the point of inoculation. Only at this time is the decay literally a black rot.

Blackening of the lesions is accompanied by a drying out of diseased tissues, with consequent shriveling and wrinkling of the cucumber. Unless secondary bacterial decay sets in, the entire fruit becomes a shriveled, blackened mummy.

Fruiting bodies (pycnidia) of the pathogen, with their exuding masses of spores (see fig. 3, *A* and *B*), constitute an important diagnostic character. Another is the mycelium of the fungus, whitish at first, which is frequently found on the surface of the lesions (see fig. 3, *B*). This develops readily under moist conditions. Thus, when many cucumbers in a crate are found with advanced lesions, the abundant mycelium becomes wet and appressed to the cucumber surface. At such times the decay may be confused with cottony leak, caused by *Pythium aphanidermatum* (Edson) Fitz.

Internal symptoms (fig. 1, *E* and *F*) are much less striking than external ones. The affected tissues are spongy soft, ranging from rather dry to fairly moist, and are frequently somewhat vacuolated. Brownish discoloration ("chamois," "buckthorn brown," and "Dresden brown") may usually be noted throughout the older areas of the lesion. The black discoloration, so conspicuous in external view, is largely confined to the rind, although an irregular blackening may occasionally be in evidence throughout portions of the diseased tissues, particularly just beneath the center of the lesion. Where infection has occurred only at the blossom end—a common occurrence on small fruits—the black, dried, decaying tip forms a conspicuous symptom.

Seen in longitudinal or cross section, the diseased tissues are irregular in outline and indefinite in extent.

No marked or specific odor is associated with the decay.

On chayote fruits the affected tissues are at first brownish. As the lesions enlarge, the surface becomes depressed, shriveled, and blackened (fig. 2, *A*). The abundant pycnidia with exuding masses of spores are a conspicuous feature. Both external and internal margins of the lesion are irregular and indefinite. The decayed tissues are brownish to black and rather shallow.

The single muskmelon fruit found with mycosphaerella black rot (fig. 2, *B*) had been infected at the blossom end. The decayed areas

were smooth and black with sharply defined borders. No fruiting bodies developed on the lesions. Internally the decayed tissues were spongy soft and black and extended well into the flesh.

The decay on Hubbard squash appeared as a large, brownish, water-soaked lesion at the blossom end. In advanced stages the rind became black and deeply wrinkled (fig. 2, *C*). Perithecia, and pycnidia with exuding spore horns, were conspicuous.

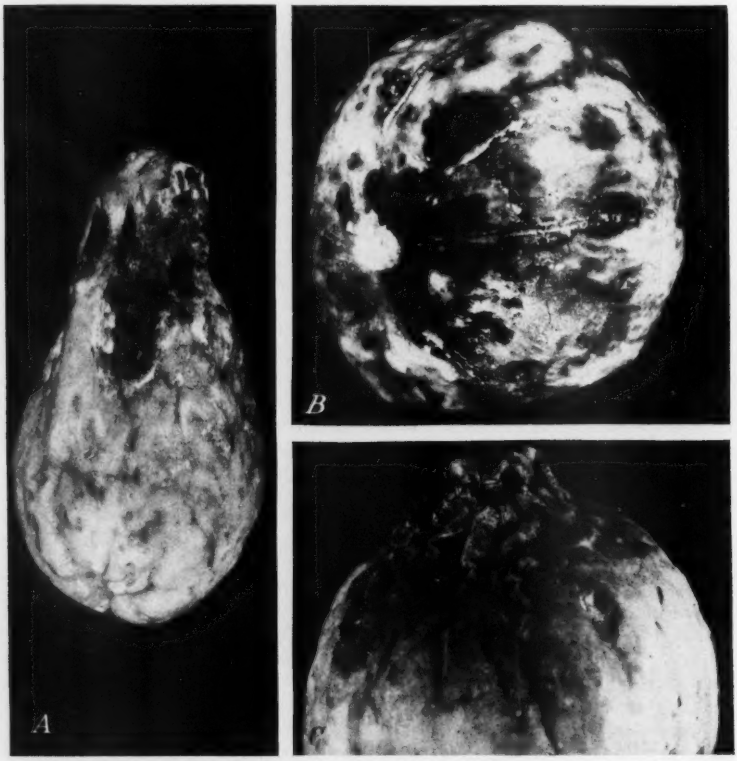

FIGURE 2.—Mycosphaerella black rot: *A*, Chayote from Jamaica, British West Indies, after 4 days at 70° to 75° F.; *B*, muskmelon from ship's stores of vessel arriving at New York, N. Y., from Venezuela; *C*, advanced lesion on Hubbard squash from Boston market.

The lesions on balsam pear were shriveled, slightly sunken, and brownish to black. The chief identifying character was the presence of the pycnidia with their conspicuous exuded spore horns.

Artificial inoculations on dishcloth gourd (*Luffa acutangula* (L.) Roxb.) yielded light-brown lesions with fairly regular but not sharply defined borders. The diseased tissues were fairly firm and slightly sunken. Here, too, the chief character for identification of the decay was the abundant pycnidial sporulation.

THE PATHOGEN

Studies were made of the pathogen in naturally occurring lesions on fruits of balsam pear, chayote, cucumber, and squash. Isolates from balsam pear, chayote, cucumber, muskmelon, squash, and watermelon were compared both in culture and in wound-inoculation lesions on cucumber fruits. Considerable variation noted in the behavior of different isolates was not found to be correlated with the types of cucurbit from which the isolates were originally obtained.

DEVELOPMENT ON CUCURBIT FRUITS

The following description of the pathogen deals primarily with its appearance on cucumber fruits in lesions that developed from both natural and artificial inoculation.

The first evidence of the pathogen is the early appearance of numerous pycnidia in the central area of the lesion. Thus mature pycnidia have been found 5 days after inoculation on cucumbers held at 70° to 75° F. At first the pycnidia are faintly brownish and entirely subepidermal. Although developed in great numbers they are at this time practically invisible to the unaided eye. Their presence is, however, readily indicated by the exuding masses of conidia that push through the broken epidermis and pile up on the surface (fig. 3, *A* and *B*). The spore masses may appear as moist, creamy, pinkish mounds or, on older, drier lesions, as firm, twisted or coiled, pinkish spore horns.

On young lesions the pycnidia have been found originating in the mesophyll just below the palisade layer and approximately 30μ to 60μ beneath the surface. More commonly they originate between the palisade cells and, by enlargement through growth, come to lie immediately beneath the epidermis.

On older lesions the abundant, dark mycelium growing throughout the rind tissues forms a semicompact layer at the surface of the cucumber fruit. The epidermis disappears, and the palisade layer becomes disorganized and is eventually destroyed. Here the pycnidia originate within the mycelium, and as they enlarge they come to lie nearly free on the surface of the lesion (fig. 3, *C*, *D*).

The pycnidia are nearly spherical though frequently narrowed toward the base (fig. 3, *D*), slightly longer than wide, and somewhat flattened at the apex, where the conidia are released through a single pore (fig. 3, *E*). Although light brownish at first, the pycnidia later darken and become nearly black. They vary greatly in size within a usual range in diameter of 109μ to 160μ. Two pycnidia were noted with diameters of 179μ and 207μ. All measurements were made from cucumber lesions resulting both from natural infections and from artificial inoculations with cucumber and squash isolates.

The conidia are produced in great abundance and emerge from the pycnidia in quantity, forming long, gelatinous spore horns. The release of the conidia can readily be observed under the lower power of the microscope (fig. 4, *A*).

The conidia are hyaline, either nonseptate or uniseptate, with rounded ends, and are somewhat constricted at the septa (fig. 4, *B* and *C*). Rarely does one observe a biseptate conidium. Considerable variation exists in the relative abundance of nonseptate and uniseptate spores.

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There was marked uniformity in the size of conidia from different sources. The following measurements are based on spores from natural infections on balsam pear, chayote, cucumber, and squash; and from

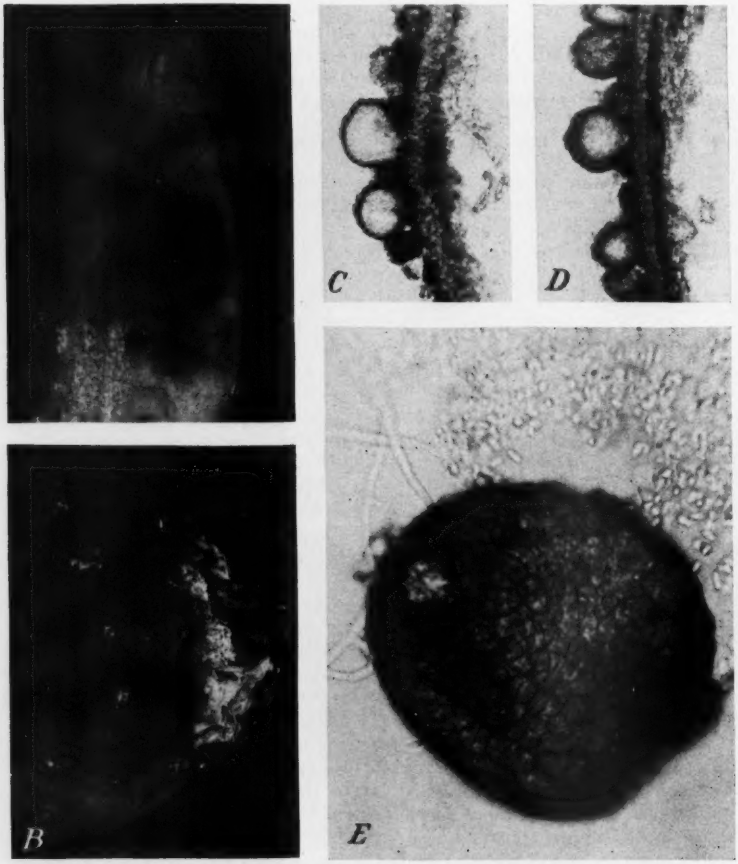

FIGURE 3.—*Mycosphaerella citrullina* on cucumber: *A*, Conidial masses first appearing on light-colored lesion; *B*, pycnidia and exuded masses of conidia found in great abundance over most of advanced lesion, with whitish mycelium developed over part of lesion; *C* and D, transverse freehand section through diseased rind, showing superficial pycnidia with palisade layer and portion of mesophyll beneath, × 95; *E*, single pycnidium, showing apical pore, × 430.

cucumbers artificially inoculated with isolates from cucumber, muskmelon, squash, and watermelon.

Nonseptate conidia averaged 4.0μ by 7.7μ, with a range of 2.8μ to 5.5μ by 4.6μ to 11.0μ. Approximately 85 percent of the spores fell within the limits of 3.7μ to 5.0μ by 5.5μ to 9.6μ. Uniseptate conidia averaged 4.4μ by 10.3μ, with a range of 2.8μ to 5.5μ by 7.3μ to 14.7μ. Approximately 98 percent fell within the limits of 3.7μ to 5.5μ by 8.3μ

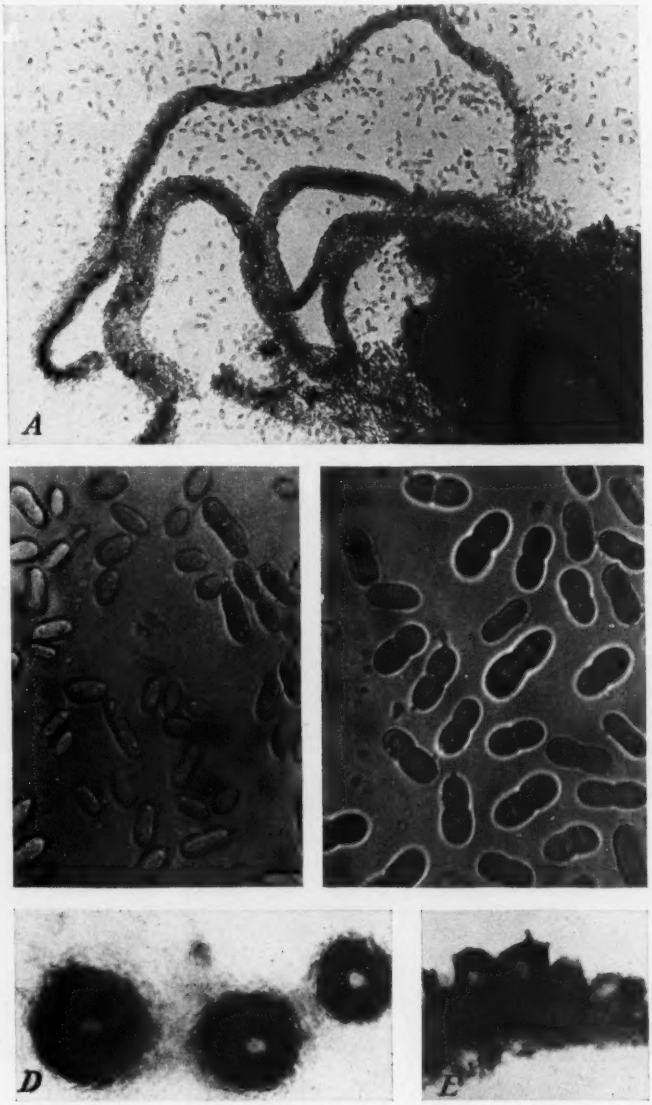

FIGURE 4.—*Mycosphaerella citrullina: A*, Two pycnidia with conidia exuded in prominent spore chains, × 95; *B*, conidia, mostly nonseptate, × 900; *C*, conidia, mostly uniseptate, × 900; *D*, scattered perithecia developed beneath the epidermis near margin of black rot lesion on Hubbard squash, × 95; *E*, transverse freehand section through diseased cucumber rind, showing superficial and nearly superficial perithecia, × 95.

to 13.8μ. A limited number of biseptate spores averaged 4.5μ by 15.4μ, with a range of 4.1μ to 5.5μ by 12.8μ to 19.3μ.

Perithecia develop later than pycnidia and are ordinarily found in great numbers, together with the latter, on the older, darkened areas

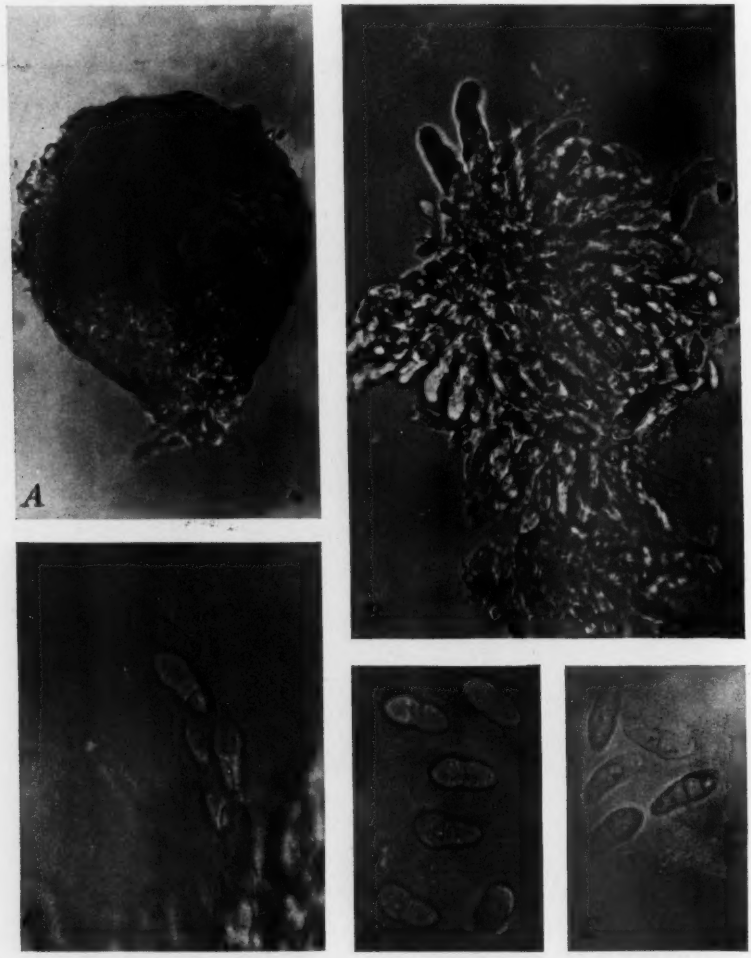

FIGURE 5.—*Mycosphaerella citrullina: A*, Single perithecium, ×430; *B*, contents of single perithecium, showing asci in compact mass, ×430; *C*, ascospores leaving ascus, ×900; *D* and *E*, free ascospores, ×900.

of the lesions. Occasionally, however, perithecia are the predominant type of fruiting structure on lesions resulting from artificial inoculation.

Single mature perithecia are occasionally observed as tiny black specks deep in the mesophyll, but they are more frequently found in the outer rind tissues only a little way beneath the epidermis (fig. 4, *D*).

On older areas of the lesion they develop in large numbers and are nearly free or have only their bases embedded (fig. 4, *E*).

The perithecia are nearly spherical to slightly elongated, with single papillate ostioles and rather heavy, nearly black walls (figs. 4, *D* and *E*, and 5, *A*). They vary greatly in size. The average diameter of those that developed on cucumber fruit lesions resulting from both natural and artificial infections was 89μ. On naturally infected chayote fruits they ranged from 91μ to 158μ with an average diameter of 124μ. On Hubbard squash lesions, the perithecia had an average diameter of 161μ and a range of 108μ to 224μ. However, isolates obtained from squash lesions, when inoculated into cucumber fruits, produced perithecia that ranged from 71μ to 110μ in diameter and averaged 87μ.

The asci tend to remain joined together at the base (fig. 5, *B*) and can best be demonstrated by partly crushing the perithecium. They are clavate-cylindrical to cylindrical and are without paraphyses. Those from cucumber lesions were found to average 10.0μ by 52.3μ with a usual range of 8.9μ to 10.6μ by 44.3μ to 76.1μ.

The asci (fig. 5, *B* and *C*) contain a single series of eight two-celled hyaline spores. The ascospores (fig. 5, *C–E*) are oblong-fusoid or fusoid, prominently constricted at the septum, with the two cells unequal. They average 6.0μ by 12.9μ, with a usual range of 4.6μ to 8.0μ by 9.2μ to 15.9μ.

DEVELOPMENT IN CULTURE

The pathogen was isolated from cucurbit fruits in Petri-dish cultures by means of tissue plantings from the advancing edge of lesions and by spore dilutions from exuded conidia. Transfers were then made from the margin of plate colonies to test-tube slants. The culture medium used was 1.5-percent potato-dextrose agar.

Although some of the isolates thus obtained eventually sporulated abundantly in culture, none did so at once, and many isolates that produced fruiting bodies only sparingly constantly remained nearly sterile. Grown on plates of potato-dextrose agar held continuously at 70° to 75° F. (fig. 6, *A*), the aerial mycelium of the poorly sporulating isolates is at first white and then gradually darkens through shades of gray to nearly black. It may range from relatively sparse to very abundant, developing uniformly or frequently with irregularly scattered cottony tufts and infrequently with slight concentric zones or faint radial lines.

Within the culture medium the mycelium is greenish black in mass and develops rapidly so that the center of the culture is prominently darkened before the plate is entirely covered by the surface mycelium. Through continued development of the substrate mycelium, the darkened area gradually extends outward to the limits of the plate, and a thin, slightly firm, felty, black fungal mat is formed at the surface of the culture medium over the entire plate. In many instances the aerial mycelium over part of the culture is appressed to the moist surface and becomes part of the mat. Frequently the fungal mat is raised and thickened at the central area of the plate. Darkening of the culture does not always proceed uniformly from the center outward but may appear in irregularly scattered areas. In all instances, however, the entire plate eventually becomes completely covered with the black fungal mat. Such fruiting bodies as may develop are found

scattered irregularly and inconspicuously over the plate and occur either singly or in tiny clusters.

Attempts were made to induce ready sporulation of these isolates by growing them on corn-meal and oatmeal agar plates and tubes, on

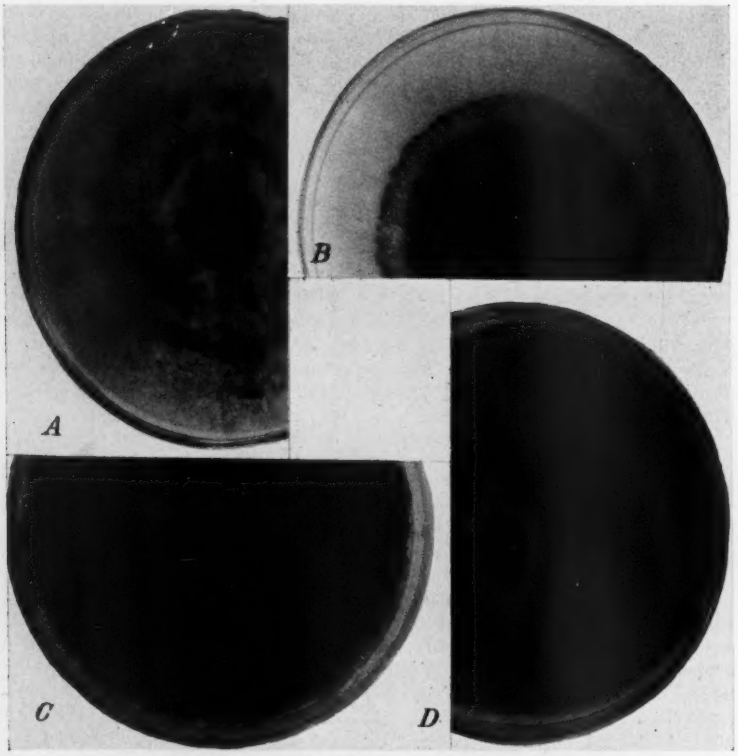

FIGURE 6.—Petri-dish cultures of *Mycosphaerella citrullina* on potato-dextrose agar. *A*, Poorly sporulating isolate from domestic Hubbard squash; aerial mycelium will eventually turn nearly black; culture 8 days old. *B*, Abundantly sporulating isolate from Puerto Rican cucumber, with sparse mycelium extending nearly to periphery of culture dish; profuse pycnidial development over entire culture, with perithecia (not distinguishable in illustration) beginning to develop near center of plate; darkening of culture has occurred in central one-half to two-thirds of plate; culture 8 days old. *C*, Abundantly sporulating isolate originally developed by sectoring from squash isolate shown in *A*; culture 14 days old, with numerous pycnidia and perithecia throughout the culture; creamy masses of exuded conidia are particularly abundant in central area. *D*, Same isolate as shown in *B*; culture 14 days old, with pycnidia and perithecia developed in abundance throughout the culture; pink masses of exuded conidia conspicuous over much of culture.

sterilized green-bean sections in tubes, sterilized cucumber rind sections in tubes and flasks, and sterilized stems of cantaloup, cucumber, squash, and watermelon, respectively, in tubes. Other tests, conducted with potato-dextrose agar as the culture medium, included cutting up plate cultures into small pieces, growing plate and tube cultures in

the dark for long periods, and exposing plate cultures to a wide range of temperatures during the course of other studies. In none of these attempts was ready sporulation induced in culture, although a few fruiting bodies similar to those produced from time to time by some of the isolates on potato-dextrose agar were occasionally observed in scattered areas of corn-meal agar and oatmeal agar plates and tubes.

During the early part of the work, those few isolates that first sporulated abundantly in culture appeared to do so more or less by chance. At that time 100 to 200 isolates were carried in culture on potato-dextrose agar plates and tubes for varying periods of time. After remaining almost completely sterile for some time, 2 of the isolates suddenly developed abundantly sporulating sectors in new plate cultures. Transfers from these sectors yielded isolates that have remained freely sporulating through many successive transfers. These, together with 20 other isolates, were then carried along as permanent stock cultures. Nearly a year and a half later 2 other isolates of the group of 22 likewise gave rise to the abundantly sporulating stage of the pathogen. Both were growing on potato-dextrose agar plates at the time. One developed a profusely sporulating sector in a plate held at 70° to 75° F. The other became moderately sporulating in the later growth that was made at 70° to 75° in plates previously held for 2 weeks at 45°. By repeated transfer from areas of the plate cultures where fruiting bodies were most abundant, the isolate eventually became profusely sporulating.

During the latter part of the work, a series of isolations was made from fruit lesions on Puerto Rican cucumbers in order to determine more definitely what proportion of the isolates would sporulate freely in culture. These were kept under observation for several weeks after transfer to tubes of potato-dextrose agar. In no case was there any evidence of abundant sporulation. After this it became necessary to set the cultures aside for 7 to 8 months; by the end of that period they had become nearly dried out. Upon resumption of the work the entire contents of the tubes were removed with a scalpel to a sterile Petri dish, and either a small piece or the entire mass was transferred to a plate of potato-dextrose agar that was held at 70° to 75° F. Within 2 to 3 weeks after transfer, 44 of the 108 isolates so tested had developed moderately to abundantly sporulating areas or sectors in the plate cultures. During subsequent transfer the abundantly sporulating stage of the pathogen was established in culture for a number of these 44 isolates. Others remained moderately sporulating, while a few never produced fruiting bodies more than sparingly.

These tests suggest that drying out of cultures favors the development of the abundantly sporulating stage of the pathogen. Observations made during the course of the work likewise indicate that once an isolate has begun to produce fruiting bodies more than sparingly it is frequently possible to bring the isolate into the completely sporulating condition by continually transferring from areas of the plate where the fruiting bodies are most abundant.

Although ordinarily there is no difficulty in maintaining an isolate in the abundantly sporulating condition once it has attained that stage, one such isolate did develop a sterile sector in a plate culture on potato-dextrose agar. Transfers from this sector have remained sterile.

On plates of potato-dextrose agar those isolates that have become abundantly sporulating in culture (fig. 6, *B–D*) produce a thin, light-colored mycelium that grows appressed to the agar. Practically no aerial mycelium develops. Pycnidia are formed early (fig. 6, *B*) and in great abundance near the surface. They are at first of light color, only slightly darker than the mycelium. Spores exuded from them pile up on the surface of the agar, giving the cultures a granular appearance. The color of the culture at this time is "light ochraceous-salmon." Before the fungus has grown to the periphery of the plate the central part of the culture becomes greenish black because of the darkening of the mycelium and pycnidia.

Perithecia, dark and somewhat smaller than the pycnidia, are slower to develop and are found first in the older, central portion of the plate. The cultures eventually become completely black (fig. 6, *C* and *D*), with inconspicuous radial or concentric patterns. Both pycnidia and perithecia (the former in greater abundance) are at this time found crowded in large numbers over the entire plate and together they constitute most of the fungus growth. Slightly pinkish masses of exuded conidia are scattered over the surface of the culture and under moist conditions may run together to form sticky or creamy spore deposits of irregular pattern. The pycnidia are for the most part similar in shape and structure to those produced in lesions on cucurbit fruits although they are somewhat smaller, falling mostly within a range of 80μ to 120μ in diameter. Larger pycnidia with two to four mouths are commonly found in smaller numbers interspersed among these. The conidia are mostly nonseptate, although a small percentage are uniseptate. In shape the nonseptate conidia are similar to but more variable than those produced on cucurbit fruits. They are also of smaller size, with a usual range of 2.1μ to 4.2μ by 5.0μ to 7.6μ, averaging 3.2μ by 5.7μ. The uniseptate conidia average 4.3μ by 9.5μ, with a usual range of 3.8μ to 5.0μ by 8.4μ to 10.9μ, and are therefore slightly smaller than those from fruit lesions.

The perithecia averaged 83μ in diameter and, although slightly smaller, were similar in shape and structure to those produced on cucurbit fruits. Both asci and ascospores were identical in size and shape with those found on fruit lesions.

In plate cultures where fruiting bodies are not produced abundantly, perithecia are frequently found in compact, somewhat spherical clusters 250μ to 450μ in diameter, with individual perithecia not clearly differentiated. It is not unusual to find asci of much greater length than normal in these clusters. Compact masses of pycnidia as large as 450μ to 850μ in diameter are likewise frequently observed in cultures that are only moderately sporulating.

IDENTITY

Chester (*8*) observed leaf spots and a vine blight of watermelon and described the fungus associated with these symptoms as *Phyllosticta citrullina* n. sp. After noting that some of the spores were uniseptate, he stated that it was questionable whether the fungus might not be classified as an *Ascochyta*. In a later paper (*9*) he reported finding perithecia of a *Sphaerella* associated with *Phyllosticta* pycnidia that developed on watermelon leaves in a moist chamber.

Only a meager description of the *Sphaerella* stage was given, and the species was not determined.

Smith (*32*) made studies of a cucurbit disease that he observed on leaves and fruit of squash and pumpkin and leaves of cucumber and cantaloup. He considered the pathogen identical with Chester's fungus, which he renamed *Ascochyta citrullina* (Chester) C. O. Sm. Smith likewise reported finding perithecia of a *Sphaerella* on stems of squash and presented considerable evidence to show that it was the perfect stage of *A. citrullina*. Although only briefly characterizing the fungus, he named it *Sphaerella citrullina* (Chester) C. O. Sm.

Grossenbacher (*12*) reported a vine wilt of greenhouse muskmelon. After comparing the pathogen with herbarium material from the Delaware Agricultural Experiment Station, he concluded that it was identical with Smith's *Sphaerella citrullina*, which he then described more completely and renamed *Mycosphaerella citrullina* (C. O. Sm.) Gross. He stated that the *Sphaerella* reported by Chester may have been the same fungus.

Grossenbacher expressed doubt concerning the relation of Chester's *Phyllosticta citrullina* to *Mycosphaerella citrullina*. He therefore redescribed the imperfect form of the latter; and, since he did not find it on the leaves and therefore not in spots, he transferred it from *Ascochyta* and renamed it *Diplodina citrullina* (C. O. Sm.) Gross.

The imperfect form of *Mycosphaerella citrullina* when producing only nonseptate conidia might readily be mistaken for a *Phyllosticta*. This has been pointed out by Chupp (*10*), who stated that there is no assurance that a distinct species of *Phyllosticta* occurs on cucurbits although several species have been described on members of this family. Longrée's observations [8] tend to support this conclusion.

In reporting a vine canker of hothouse cucumbers, Massee (*17*) identified the pathogen as *Mycosphaerella citrullina* although he had observed only the imperfect form of the fungus. On the basis of limited cross-inoculation tests he concluded that the cucumber pathogen was also responsible for a similar stem canker of greenhouse tomatoes. After the appearance of Massee's paper, a number of other British reports were made of the tomato disease under the name "melon or cucumber canker" (*19*, *20*) and "cucumber and tomato canker" (*1*, *2*, *3*, *11*). Brooks and Price (*6*), in England, and Schoevers (*29*), in Holland, made studies of the tomato disease and likewise referred the pathogen to *M. citrullina*. However, work of Klebahn (*16*) and of Brooks and Searle (*7*) showed conclusively that the tomato pathogen was identical with *Diplodina lycopersici* Hollós and was therefore distinct from the cucurbit fungus. Klebahn observed and studied the perfect stage of the tomato pathogen, which he described as *Didymella lycopersici* (Hollós) Kleb. (*16*).

Most investigators have accepted Grossenbacher's usage (*12*) although, according to Keissler (*15*), *Ascochyta citrullina* (Chester) C. O. Sm. is identical with *A. cucumis* Faut. and Roum., which was described from leaves of cucumber (*28*) during the same year that Chester (*8*) described *Phyllosticta citrullina*. Keissler likewise considered *A. melonis* Potebnia (*23*), which was found attacking all above-ground parts of muskmelon, to be identical with *A. cucumis* Faut. and Roum. Potebnia, however, found his *Ascochyta* associated

[8] LONGRÉE, K. MYCOSPHAERELLA CITRULLINA ON ORNAMENTAL GOURDS (CUCURBITA PEPO). U. S. Bur. Plant Indus., Plant Dis. Rptr. 24: 222-224. 1940. [Processed.]

with a perfect form possessing paraphyses. This he identified as *Didymella melonis* Pass. and apparently assumed that it represented the perfect stage of *A. melonis*.

During the present studies it was found that the cucumber pathogen was identical with that isolated from domestic squash affected by black rot. Although some variation in the size of spores and fruiting bodies was noted between the measurements for both the cucumber and the squash pathogen and those reported for *Mycosphaerella citrullina* by Grossenbacher (*12*), Hemmi (*13*), and Longrée,[9] the differences were not great. The fungus that was found throughout the course of these studies to be responsible for the decay of West Indian cucurbit fruits is therefore considered to be *M. citrullina*.

TEMPERATURE RELATIONS

Occasionally in reports on the prevalence of gummy stem blight of cucumbers and melons the statement is made that the disease is favored by high temperatures. Kauffman (*14*) found that the pathogen from squash made only slow growth on corn-meal agar when held mostly at temperatures of 4° to 8° C., with an occasional upward range to 10°. These appear to be the only references in the literature to the relation of temperature to the growth of the pathogen or the development of the disease.

A study was therefore made on the effect of temperature on the growth of *Mycosphaerella citrullina* in Petri dishes containing potato-dextrose agar. In each test five cultures were used at each temperature. The diameters of the colonies were measured after 4 days at temperatures ranging by 5° intervals from 35° to 95° F; after 1 week at 35° to 60°; and after 2 weeks at 35° to 50°. Ten such tests were conducted; in each a different isolate was employed. These included seven obtained from Puerto Rican and Cuban cucumbers, two from Puerto Rican chayote, and one from domestic Hubbard squash.

The average results of the 10 tests are shown in figure 7, *A* to *C*. At the end of 4 days no growth had occurred at either 35° or 40° F. Slight growth had taken place at 45° and an increasing rate of growth was noted with increase of temperature from 45° to 80°. Maximum growth occurred at 80°. Growth fell off rapidly from 80° to 90°, with little growth at the latter temperature. No growth occurred at 95°.

No growth was found at 35° F. after 1 week. At 40° there was slight growth and at 45° slight to moderate growth. The rate of growth increased rapidly with increase of temperature from 50° to 60°. At the end of 2 weeks no growth had occurred at 35°, slight growth had taken place at 40°, moderate growth at 45°, and extensive growth at 50°. Cultures held at 95° showed no growth in 2 weeks; cultures at 35° showed no growth after 6 weeks.

The effect of temperature on the development of decay was determined by holding inoculated cucumbers at a series of temperatures ranging by 5° intervals from 35° to 65° F. The cucumber fruits were disinfected by immersing them for 30 minutes in a 1 : 1,000 solution of mercuric chloride; they were then rinsed in sterile water and placed in sterilized moist chambers. The inoculum consisted of small blocks

[9] See footnote 8, p. 207.

of 2-week-old potato-dextrose agar plate cultures of the cucumber pathogen. Wound inoculations were made at two points on each of the eight cucumbers used at each temperature of a given test. Half of the cucumbers were removed at the end of 1 week and half at the end of 2 weeks. Measurements of the diameters of the decay lesions were made after cutting the cucumbers transversely at the point of inoculation. The results obtained by averaging six such tests are shown in figure 7, *D* and *E*.

At the end of 1 week there was no development of decay at either 35° or 40° F. Decay was slight at 45° and moderate at 50°. From

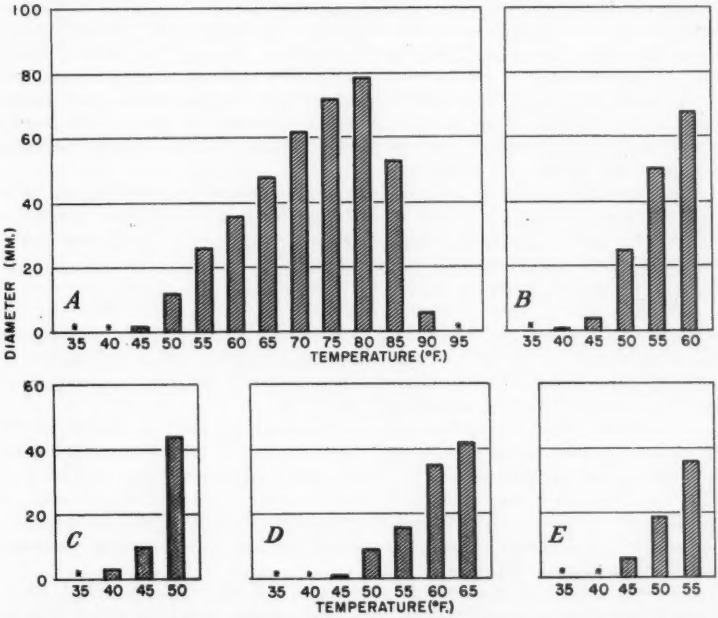

FIGURE 7.—*A–C*, Temperature relations of *Mycosphaerella citrullina* as determined by average diameter of 50 colonies on potato-dextrose agar after (*A*) 4 days, (*B*) 1 week, and (*C*) 2 weeks; *D* and *E*, temperature relations of mycosphaerella black rot as determined by average diameter of 48 lesions on cucumber fruits 1 week (*D*) and 2 weeks (*E*) after inoculation. Asterisk (*) indicates that there was no growth for the particular period.

50° to 65° the rate of decay increased rapidly with increase of temperature, so that by the end of 1 week the lesions were 42 mm. in diameter at 65°.

Two weeks after inoculation decay had not developed at 35° or 40° F., although moderate-sized lesions were evident at 45°. Rapid increase in decay occurred at 50° and 55°, so that lesions at 55° were 36 mm. in diameter.

In other tests advanced lesions placed at low temperatures showed no further enlargement at 35° F. after 2 weeks and very slight enlargement after 1 week at 40°.

CONTROL

Observations made during the present study suggest that much of the mycosphaerella black rot found in Puerto Rican and Cuban cucumbers on the market had its origin in field infections. Control of the disease in the field is essential in any program for its control on the market. Seed treatments, because the pathogen is seed-borne, and the application of bordeaux mixture spray or copper-lime dust are the chief field control measures recommended for cucumbers (*37*) and other cucurbits (*34*).

During the course of the market studies it was found that cucumber fruits could readily be inoculated with *Mycosphaerella citrullina* at skin breaks and wounds. Although the tests were somewhat limited, in no case was there any evidence of infection having occurred through the unbroken skin of mature fruits. Early tests by Meier[10] indicated that infection could be accomplished by puncturing sound cucumbers with one's fingernail while handling cucumbers on which the fungus was fruiting. Therefore, care should be taken to avoid rough handling during harvesting and packing.

No experiments have been conducted to determine how much infection occurs during the picking and packing operations. However, the rapid and abundant production of spores on black rot lesions suggests that many cucumbers harvested from diseased fields might well be expected to carry a heavy spore load.

Distribution of spores over the surface of cucumbers could readily occur during washing operations, particularly if diseased cucumbers are overlooked and thus reach the wash water. The possibility of control through adding fungicides to the wash water or through the use of a fungicidal bath should be studied.

A number of investigators have pointed out that in the field the disease is usually more serious and spreads more rapidly when moisture is high. Cucumbers that are harvested during rainy periods or shortly thereafter are therefore more subject to decay during the marketing period. Regardless of conditions prevailing in the field, cucumbers and other curcurbits should be moved to market as soon as possible after being harvested.

One of the most important measures for reducing market losses from mycosphaerella black rot in cucumbers shipped from Puerto Rico and Cuba is to precool them to 50° F. or below. The rapidity with which the decay develops between 55°–85° emphasizes the importance of prompt cooling. The results of the temperature studies show definitely that the decay can be almost completely held in check at 45°, although a carrying temperature of 40° would be preferable. In this connection it should be remembered that prolonged exposure of cucumbers to temperatures of 45° or lower may cause low-temperature break-down (*24*, *27*). However, for short transit periods of 1 week or less there is probably little danger of low-temperature injury at 40°–45°, the temperatures here recommended for Puerto Rican and Cuban cucumbers.

SUMMARY

During investigations extending from 1938 to 1942, inclusive, mycosphaerella black rot, caused by the fungus *Mycosphaerella ci-*

[10] See pp. 251 and 252 of reference in footnote 4, p. 193.

trullina (*C. O. Sm.*) Gross., was found to be chiefly responsible for the serious losses from decay frequently observed in Puerto Rican and Cuban cucumbers arriving on the New York, N. Y., market.

A review of the literature shows that the pathogen is widely distributed and attacks many cucurbits of economic importance. Symptoms as reported may include spotting of the leaves; production of cankers on the stems, petioles, and fruitstalks; decay of the stem; wilt of the vine; and rotting of the fruit.

Although market losses were greatest on Puerto Rican and Cuban cucumbers, the decay was also found affecting balsam pear, chayote, Chinese preserving melon, Yellow Crookneck squash, and watermelon from Cuba; chayote from Jamaica and Puerto Rico, and muskmelon presumably from Venezuela. During extensive observations on the New York market the decay was noted only once on domestic cucumbers (in a carlot from Georgia), and in no instance was it found on any type of domestic muskmelon. The decay came to attention twice on domestic Hubbard squash (from Virginia and from Massachusetts) but it was not observed on domestic watermelon during the course of limited observations.

Symptoms of mycosphaerella black rot on cucumbers are described and illustrated in detail and, to a lesser extent, symptoms on balsam pear, chayote, dishcloth gourd, muskmelon, and squash.

An illustrated account is given of the development of the pathogen on cucurbit fruits and its cultural behavior on natural and artificial media.

Temperature-relation studies showed that the pathogen makes most rapid growth in culture at 65° to 85° F., with the maximum at 80°. No growth occurred at 95° even in 2 weeks. Growth was markedly reduced at 45°, while only slight growth took place in 1 to 2 weeks at 40°. No growth occurred at 35° in 6 weeks.

The development of decay on cucumber fruits was very slow at 45° F. No decay took place at 35° or 40°. Rapid increase in decay occurred at 50° and 55°.

Measures suggested for reducing market losses (particularly for Puerto Rican and Cuban cucumbers) include field control through seed treatment and spraying, care in picking and packing to avoid mechanical injuries, prompt handling after picking, precooling to 50° F. or somewhat lower, and maintaining temperatures of 40° to 45° during the period of transit to the market.

LITERATURE CITED

(1) Anonymous.
1909. cucumber and tomato canker. [Gt. Brit.] Min. Agr. and Fisheries Jour. 16: 579–581.

(2) ———
1910. cucumber and tomato canker. [Gt. Brit.] Min. Agr. and Fisheries Jour. 17: 216–217.

(3) ———
1913. destructive insects and pests scheduled by the board of agriculture and fisheries—xiii. cucumber and tomato canker. Gard. Chron. (ser. 3) 54: 167–168, illus.

(4) Alvarez, L. A.
1940. mycosphaerella rot caused damage of cucumber fruits in transit. Puerto Rico Univ. (Rio Piedras) Agr. Expt. Sta. Ann. Rpt. 1939–40: 47.

(5) Boyd, O. C.
1932. PLANT DISEASES OBSERVED IN 1931, NOT HERETOFORE REPORTED IN MASSACHUSETTS. Mass. Agr. Expt. Sta. Bul. 280 (Ann. Rpt. 1931), p. 207.

(6) Brooks, F. T., and Price, S. R.
1913. A DISEASE OF TOMATOES. New Phytol. 12: 13–21, illus.

(7) ——— and Searle, G. O.
1921. AN INVESTIGATION OF SOME TOMATO DISEASES. Brit. Mycol. Soc. Trans. (1920) 7: 173–197.

(8) Chester, F. D.
1891. NOTES ON THREE NEW OR NOTEWORTHY DISEASES OF PLANTS. Torrey Bot. Club Bul. 18: 371–374.

(9) ———
[1893.] REPORT OF THE MYCOLOGIST. Del. Agr. Expt. Sta. Ann. Rpt. 5 (1892): 53–80, illus.

(10) Chupp, C.
1925. MANUAL OF VEGETABLE-GARDEN DISEASES. 647 pp., illus. New York.

(11) Collinge, W. E.
1911. THE WILT DISEASE OF TOMATOES AND CUCUMBERS. First Report on Economic Biology, Birmingham [England], pp. 45–46.

(12) Grossenbacher, J. G.
1909. A MYCOSPHAERELLA WILT OF MELONS. N. Y. State Agr. Expt. Sta. Tech. Bul. 9, pp. [191]–229, illus.

(13) Hemmi, T.
1922. ON THE OCCURRENCE OF MYCOSPHAERELLA WILT OF MUSKMELONS IN JAPAN. Phytopathology 12: [394]–397.

(14) Kauffman, C. H.
1921. A BLACK ROT OF SQUASH. Mich. Acad. Sci., Ann. Rpt. 22: [201]–202.

(15) Keissler, K.
1923. REVISION EINIGER VON FAUTREY AUFGESTELLTER PILZE. Ann. Mycol. 21: [70]–83.

(16) Klebahn, H.
1921. DER PILZ DER TOMATENSTENGELKRANKHEIT UND SEINE SCHLAUCHFRUCHTFORM. Ztschr. f. Pflanzenkrank. 31: 1–16, illus.

(17) Massee, G.
1909. XXXVIII.—CUCUMBER AND TOMATO CANKER, (MYCOSPHAERELLA CITRULLINA, GROSSENB.) Kew Roy. Bot. Gard. Bul. Misc. Inform. 7: 292–293, illus.

(18) Meier, F., Drechsler, C., and Eddy, E.
1922. CUCUMBER BLACK ROT CAUSED BY MYCOSPHAERELLA CITRULLINA. (Abstract) Phytopathology 12: 43.

(19) Middleton, T. H.
1911. MELON OR CUCUMBER CANKER. [Gt. Brit.] Bd. Agr. and Fisheries, Intel. Div., Ann. Rpt. 1909–10 (2): 62.

(20) ———
1911. MELON OR CUCUMBER CANKER. [Gt. Brit.] Bd. Agr. and Fisheries, Intel. Div., Ann. Rpt. 1910–11 (2): 54–56.

(21) Orton, W. A.
1917. WATERMELON DISEASES. U. S. Dept. Agr. Farmers' Bul. 821, 18 pp., illus.

(22) ——— and Meier, F. C.
[1922.] DISEASES OF WATERMELONS. U. S. Dept. Agr. Farmers' Bul. 1277, 31 pp., illus.

(23) Potebnia, A.
1910. BEITRÄGE ZUR MICROMYCETENFLORA MITTEL-RUSSLANDS. Ann. Mycol. 8: 42–93, illus.

(24) Ramsey, G. B., Wiant, J. S., and Link, G. K. K.
1938. MARKET DISEASES OF FRUITS AND VEGETABLES: CRUCIFERS AND CUCURBITS. U. S. Dept. Agr. Misc. Pub. 292, 74 pp., illus.

(25) Ridgway, R.
1912. Color standards and color nomenclature. 43 pp., illus. Washington, D. C.

(26) RODIGHIN, (M. [N.])
1939. O REDKOI BOLEZNI TYKVI I KABACHKOV, VYZYVAEMOI ASCOCHYTA CITRULLINA (C. O. SMITH) GROSS. [On a rare disease of vegetable marrow and Italian marrow, caused by Ascochyta citrullina (C. O. Smith) Gross.] Saratovsk. Selsk. Khoz. Inst. Izv. (Inst. Agron, Saratov, Ann.) 6:191–194. (Abstract) Rev. Appl. Mycol. 19: 66, 1940. [Original not seen.]

(27) ROSE, D. H., WRIGHT, R. C., and WHITEMAN, T. M.
1938. THE COMMERCIAL STORAGE OF FRUITS, VEGETABLES, AND FLORISTS' STOCKS. U. S. Dept. Agr. Cir. 278, 52 pp. (Revised.)

(28) ROUMEGÙERE, C.
1891. FUNGI EXSICCATI PRECIPUE GALLICI. Rev. Mycol. 13: 73–83.

(29) SCHOEVERS, T. A. C.
1919. DE TOMATENKANKER, EEN VOOR NEDERLAND NIEUWE, ERNSTIGE TOMATENZIEKTE. Tijdschr. over Plantenziekten 25: [174]–192, illus.

(30) SHERBAKOFF, C. D.
1917. SOME IMPORTANT DISEASES OF TRUCK CROPS IN FLORIDA. Fla. Agr. Expt. Sta. Bul. 139, pp. [191]–277, illus.

(31) ———
1920. REPORT OF TRUCK PATHOLOGIST. Fla. Agr. Expt. Sta. Ann. Rpt. 1920: 40R–45R.

(32) SMITH, C. O.
1905. THE STUDY OF THE DISEASES OF SOME TRUCK CROPS IN DELAWARE. Del. Agr. Expt. Sta. Bul. 70, 16 pp., illus.

(33) WALKER, M. N.
1936. INVESTIGATION OF AND CONTROL OF FUNGOUS DISEASES OF WATERMELONS. Fla. Agr. Expt. Sta. Ann. Rpt. 1936, p. 88.

(34) ——— and WEBER, G. F.
1931. DISEASES OF WATERMELONS IN FLORIDA. Fla. Agr. Expt. Sta. Bul. 225, 52 pp., illus.

(35) WARDLAW, C. W., LEONARD, E. R., and BAKER, R. E. D.
1934. OBSERVATIONS ON THE STORAGE OF VARIOUS FRUITS AND VEGETABLES. I.—TOMATOES, CAULIFLOWERS, STRING BEANS, EGGPLANT, CUCUMBERS, and MUSKMELONS. Trop. Agr. [Trinidad] 11: 196–200, illus.

(36) WEBER, G. F.
1925. DISEASES OF CUCUMBERS. Fla. Agr. Expt. Sta. Bul. 177, pp. [25]–71, illus.

(37) ———
1929. CUCUMBER DISEASES IN FLORIDA. Fla. Agr. Expt. Sta. Bul. 208, 48 pp., illus.

EMBRYOLOGY OF THE TUNG TREE[1]

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INTRODUCTION

The initiation of the pistillate flower, the development of the ovary, the growth of the fruit and its parts, and the general aspects of the morphology and anatomy of the fruit of the tung tree (*Aleurites fordi* Hemsl.) have been reported in earlier papers (*1*, *3*, *5*, *6*).[2] However, cytological details of megasporogenesis, embryo sac formation, and embryo growth have not been described, and it is the purpose of this paper to supplement the earlier papers with this information.

REVIEW OF LITERATURE

Botanical information pertaining to the genus *Aleurites* is limited to a few publications concerned with elementary descriptive morphology. To date there has been no attempt to study the cytology of the genus. However, considerable data have been published on species of various other genera in the family Euphorbiaceae. Modilewski (*7*, *8*, *9*) has described embryo sac development in several species of *Euphorbia*. He reported that the ovule of *E. procera* has a projected nucellus, which is in direct contact with papillate cells of a specialized obturator. He further stated that the ovules of most Euphorbiaceae contain a single megaspore cell that divides to form four daughter cells, of which the chalazal cell develops a normal eight-nucleate embryo sac. Lyon (*4*) likewise reported a projected nucellus in *E. corollata*, and Schlotterbeck (*12*) described a similar structure in *Croton tiglium*. These structures are to be compared with the similar structure found in tung.

According to a summary of types of embryo sac development in the Euphorbiaceae by Schnarf (*13*, *pp. 220–221*), the majority of the species of that family have a normal eight-nucleate embryo sac development.

D'Amato (*2*) reported that in *Euphorbia paralias*, *E. falcata* var. *acuminata*, *E. pubescens*, *E. pithyusa* var. *ovalifolia*, *E. characias*, and *E. amygdaloides* the archesporium is usually unicellular, that the female gametophyte is produced by a chalazal megaspore, and that

[1] Received for publication October 30, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 229.

most species show the *Scilla* type of gametophyte either regularly or occasionally. The fertilized egg usually rests, while the triple fusion nucleus immediately divides to give rise to the endosperm. Antipodals degenerate early.

A study of the pistillate flower and developing fruit of tung by McCann (*5*) included the general aspects of the morphology of the ovary and its parts and the growth of these parts into the mature fruit and seed. The gross and the vascular anatomy of the fruit and seed were discussed. The development of the pistillate flower begins 5 to 10 months before anthesis. The primordia may be initiated at any time between late May and late October. All pistillate buds develop to the characteristic cup stage, consisting mainly of carpellary tissue, before the winter months. There is no development of the placenta and septa until the renewal of growth in the spring, usually about the first part of February. The first indication of resumed growth in the pistil is observed in the edges of the carpellary cup; about the same time the axial placentae begin to develop as mounds of tissue rising in the base of the cup. Locular partitions, or septa, rise between the incipient locules and connect the ovary wall to the placentae. The placentae, septa, and ovary wall eventually converge at the apex and close over the locules. The ovary at anthesis is superior and polycarpellary, with axial placentae.

METHODS AND MATERIALS

From September 1938 to June 1942 terminal buds, flowers, and fruits were collected from tung trees within a 25-mile radius of Bogalusa, La., and were fixed in a variety of fixatives. Beginning January 1, 1939, weekly collections were made from each of thirty 5- to 12-year-old seedling trees selected for their variation in habitat and growth characteristics. At least 3 buds were taken from each tree at weekly intervals through December 1939. In addition, during each collection period 30 to 50 buds were collected at random from trees in the immediate vicinity of the selected seedlings.

During 1940 monthly collections were made from miscellaneous trees except from May to October, when collections were made at weekly intervals. These collections were studied by means of a dissection technique (*6*).

During the flowering periods of 1939–41 hand-pollinations were made and after pollination fruits were collected at approximately 12-hour intervals for 1 week, then at 24-hour intervals for 2 weeks, and at weekly intervals for 2½ months.

Pistillate flowers were dissected from the terminal buds collected during the dormant period up to anthesis, dehydrated in a series of ethyl and butyl alcohols, embedded in paraffin, and sectioned on a rotary microtome at 8μ to 15μ. Individual ovules were dissected from fruits collected after anthesis. These were treated in the same manner as the pistillate buds. Some whole fruits collected 6 weeks after pollination were sectioned either as a whole or in part.

Sections were stained in safranine 0 and fast green, crystal violet, iron-alum haematoxylin, and crystal violet and eosin.

Photomicrographs were made with 16-, 32-, 48-, and 72-mm. Micro Tessar lenses and with 2-, 4-, and 8-mm. apochromatic objectives in

combination with 10 × or 15 × compensating oculars. A bellows extension was used in making all photographs.

Drawings were made with the aid of a camera lucida or by direct tracing of enlarged photographs.

OBSERVATIONAL DATA

INITIATION OF OVULE

Ovule primordia usually make their appearance in late February, at the time or slightly before the locules are closed over by growth of the carpel walls. The ovule primordia appear on the axial placenta near the apical end of each locule and consist of two integuments surrounding a large nucellar tissue. The primordia develop in the manner typical of the normal anatropous ovule except that, in the very early stages of primordia initiation, the nucellus protrudes through and beyond the micropylar opening formed by the integuments. The protruding nucellus is also typical of other Euphorbiaceae (*10, pp. 184–189*).

MEGASPOROGENESIS

About 2 weeks before anthesis the megaspore mother cell is differentiated from a hypodermal cell of the nucellus (fig. 1, *A*, *B*). At this time it is located near the apex and is readily distinguishable from other cells of the nucellus by its enlarged nucleus, elongated appearance, and rather extensive vacuolization. As the megaspore elongates in preparation for division, it becomes more deeply imbedded, so that, by the time the nucleus divides, the cell lies at the approximate center of the ovule (fig. 1, *C*, *D*). Two transverse divisions take place, giving rise to a row of four megaspores, the outer three of which degenerate (fig. 2, *A–D*). The chalazal megaspore enlarges and elongates in preparation for the third division during which two nuclei form; one of these orients itself in the micropylar end and the other in the chalazal end of the embryo sac (figs. 1, *E*, *F*, and 2, *D*, *E*). The fourth division is periclinal, giving rise to two nuclei at each pole (fig. 2, *F*). Each nucleus of the sac then goes through the fifth and final division to form eight nuclei (fig. 2, *G*).

The nuclei then orient themselves in the conventional manner of the normal eight-celled embryo sac, consisting of an egg and two synergids in the micropylar end, three antipodals in the chalazal end, and two polar nuclei at or near the center of the sac (fig. 2, *H*).

FERTILIZATION OF EGG AND GROWTH OF EMBRYO

The egg nucleus is fertilized between 24 and 36 hours after pollination. This was made evident by the presence of discharged pollen tubes in the embryo sac, enlarged and more darkly staining egg nuclei, vacuolization of the egg cell cytoplasm, and in a few cases the presence of the male nucleus within the egg cell and in contact with the egg nucleus (fig. 1, *H*, *I*).

Synergids degenerate early, sometimes even before fertilization. The antipodals are also short-lived and usually show signs of degeneration at the time of fertilization or soon after (figs. 1, *G*, and 2, *I*).

The fertilized egg does not immediately begin to divide but rests for 1 to 2 weeks. The earliest division of the fertilized egg was ob-

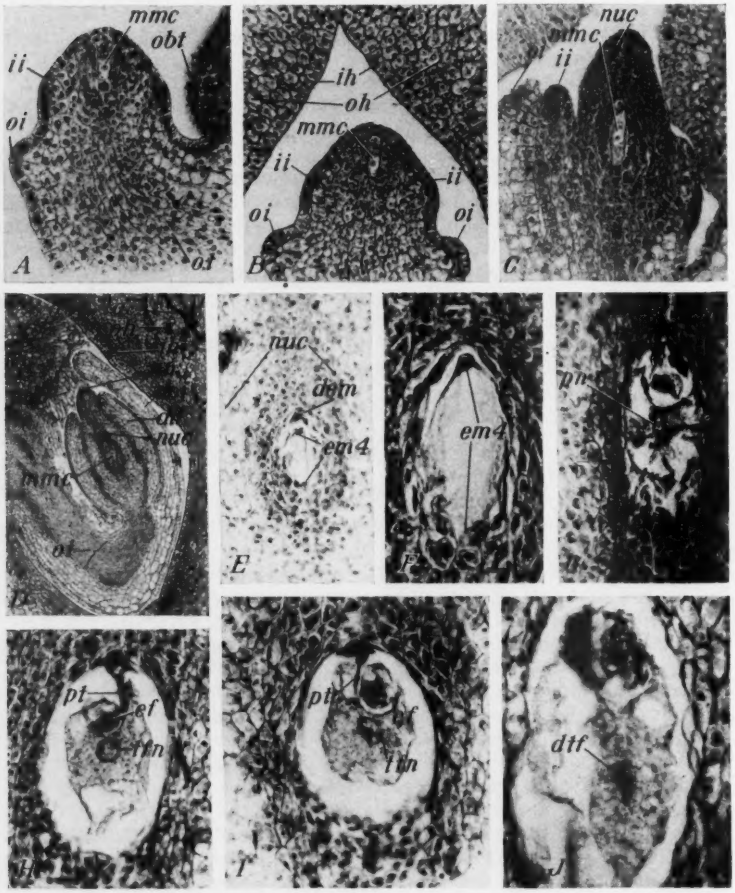

FIGURE 1.—*A*, Median radial longitudinal section of young ovule, showing megaspore mother cell and integument primordia; March 21, 1941. *B*, Median tangential longitudinal section, showing megaspore mother cell and portion of ovary wall; March 19, 1941. *C*, Longitudinal section of ovule, showing growth of integument primordia and nucellus and elongation of megaspore mother cell in preparation for first division; March 28, 1941. *D*, Median radial longitudinal section of ovule, showing integuments, nucellar neck, obturator and its papillate cells, ovular trace, and the beginning of the development of the inner hull from the inner layers of the ovary wall; April 6, 1941. *E*, Longitudinal section through ovule, showing three degenerating megaspores and two nuclei, one at each pole of embryo sac; nuclei have resulted from division of the chalaza of the four original megaspores; April 3, 1941. *F*, Four-nucleate embryo sac in which three of the nuclei are visible, two in the chalazal end and one in the micropylar end of the sac; March 28, 1941. *G*, Longitudinal section through embryo sac, showing fusion of polar nuclei in center; early degeneration of synergid cell is apparent in micropylar end of sac; April 3, 1941. *H*, Embryo sac showing presence of discharged pollen tube lying adjacent to the egg cell, 2*n* endosperm nucleus (possibly 3*n*), and two of the three degenerating antipodals; April 6, 1941. *I*, Embryo sac in approximately same stage as shown in *H*; nuclei of both egg and endosperm appear to have been fertilized by generative nuclei; April 6, 1941. *J*, Embryo sac showing first division of the 3*n* endosperm nucleus, 52 hours after pollination; April 8, 1941. *A*, *B*, *E*, and *G*, × 165; *C*, × 160; *D*, × 65; *F*, × 350; *H* and *I*, × 185; *J*, × 340. *dem*, Degenerating megaspores; *dii*, dorsal inner integument; *doi*, dorsal outer integument; *dtf*, first division of triple fusion nucleus; *ef*, fertilized egg; *em4*, four-celled embryo sac; *ih*, inner hull; *ii*, inner integument; *lac*, latex cells; *mmc*, megaspore mother cell; *nn*, nucellar neck; *nuc*, nucellus; *obt*, obturator; *oh*, outer hull; *oi*, outer integument; *ot*, ovular trace; *pn*, polar nucleus fusing; *pap*, papillate cells of obturator; *pt*, pollen tube; *tfn*, triple fusion nucleus.

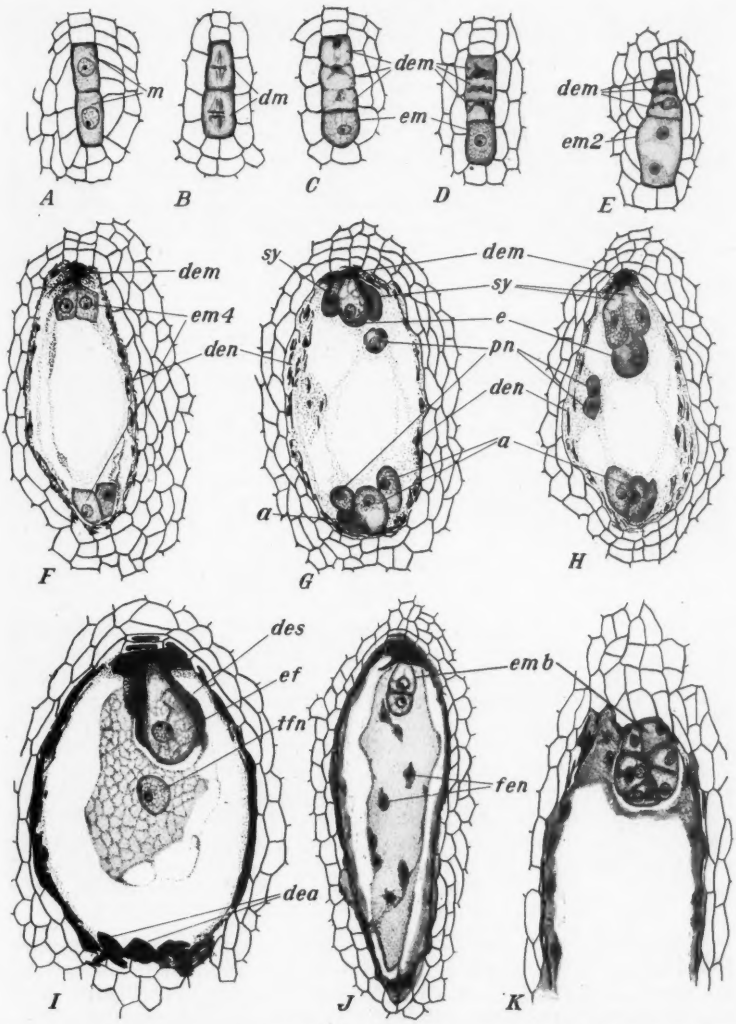

FIGURE 2.—*A*, Two macrospores resulting from the first division of the macrospore mother cell; March 28, 1942. *B*, Two macrospores undergoing the second division in the formation of the embryo sac; March 30, 1941. *C*, Row of four macrospores, the upper three showing some degeneration of cellular contents; April 2, 1941. *D*, Further degeneration of upper three macrospores and enlargement of chalazal macrospore in preparation for the third division in the formation of the embryo sac; April 3, 1941. *E*, Two nuclei resulting from the third division, and three degenerating micropylar macrospores; April 6, 1941. *F*, Four-celled embryo sac resulting from the fourth division; two cells are oriented in the micropylar end and two in the chalazal end of the sac; April 8, 1941. *G*, Eight-celled embryo sac resulting from fifth and final division; April 9, 1941. *H*, Orientation of embryo sac nuclei in normal manner; an egg and two synergids in the micropylar end, three antipodals in the chalazal end, and two polar nuclei at or near the center of the sac; April 10, 1941. *I*, Fertilized eggs, degenerated synergids and antipodals, and triple fusion nucleus; April 27, 1941. *J*, First division of the fertilized egg surrounded by free endosperm nuclei; May 2, 1941. *K*, Eight-celled embryo surrounded by peripheral band of free endosperm nuclei; May 15, 1941. Drawings made with the aid of a camera lucida. *A* to *H*, × 390; *I* and *K*, × 170; *J*, × 150. *a*, Antipodals; *dea*, degenerating antipodals; *dem*, degenerating macrospores; *den*, degenerating nucellus; *des*, degenerating synergids; *dm*, dividing megaspores; *e*, egg; *ef*, fertilized egg; *em*, megaspore-forming embryo sac nuclei; *em2*, first two-embryo sac nuclei; *em4*, four-embryo sac nuclei; *emb*, embryo; *fen*, free endosperm nuclei; *m*, megaspores; *pn*, polar nucleus; *sy*, synergid; *tfn*, triple fusion nucleus.

served at 120 hours after pollination. The next few divisions of the embryo take place comparatively slowly. Embryos collected 2 to 4 weeks after pollination were found in the 8-cell stage; at 6, 7½, and 10 weeks after pollination, most embryos were in the 32-cell stage. Thus it appears that the embryo may rest for a month or more at the 32-cell stage. This rest period usually comes during the latter part of May or early June, depending on the season.

The first division of the fertilized egg is in a transverse plane (fig. 2, *J*). The subsequent divisions have not been seen, but they are such as to cause the embryo to assume a spheroid shape at the 32- to 64-cell stages.

SUSPENSOR

The suspensor of the tung embryo is multicellular (fig. 3, *I*). A study of the shape and position of the cells in the four- and eight-celled embryos indicates that the suspensor may develop from the upper of the first two cells resulting from the egg cell divisions. The suspensor is attached to the lower part of the nucellar neck, which extends through the micropyle (fig. 3, *C*, *D*, *F–H*). In the early stages of development, the connecting cells are large and thin-walled at the point of attachment. By the time the embryo begins rapid growth, usually about July 1, the suspensor has developed into a multicellular vermiform structure (fig. 3, *J*).

DEVELOPMENT OF ENDOSPERM

The two polar nuclei fuse just before their union with the second male nucleus, forming the $3n$ fusion nucleus. The first division of this $3n$ nucleus was observed in material collected 52 hours after pollination (fig. 1, *J*). At 62 hours after pollination, eight free endosperm nuclei were found. Subsequent divisions take place regularly but relatively slowly. By the first of June the endosperm nuclei, which are embedded in heavy protoplasm, form a single layer around the periphery of the embryo sac. In 1941 the first evidence of multiple layers of endosperm was observed on June 6. Numerous division figures were found in the micropylar end of the sac in the vicinity of the embryo (fig. 3, *E*). Cell walls began to form in the endosperm about 10 days later.

PERIOD OF RAPID DEVELOPMENT OF EMBRYO AND ENDOSPERM

About July 1 both endosperm and embryo begin to develop rapidly. Cotyledon primordia appear and rapidly extend through the embryo sac (fig. 3, *I–K*). The endosperm tissue develops centrifugally from the peripheral layer around the embryo sac and by late August fills the seed at the expense of the nucellus and inner integument tissue.

Since only the outer epidermis of the inner integument is concerned in the development of the shell, the role of the remaining tissue of the inner integument must be considered in relation to the developing embryo and endosperm. The nucellus occupies the center of the young ovule with the exception of the space occupied by the embryo sac. The nucellus is enclosed b yinner integument tissue. In the chalazal end of the ovule the nucellus is separated from the inner integument by the conducting tissue that extends from the ovular trace through the opening in the chalazal end of the seed coat. In the

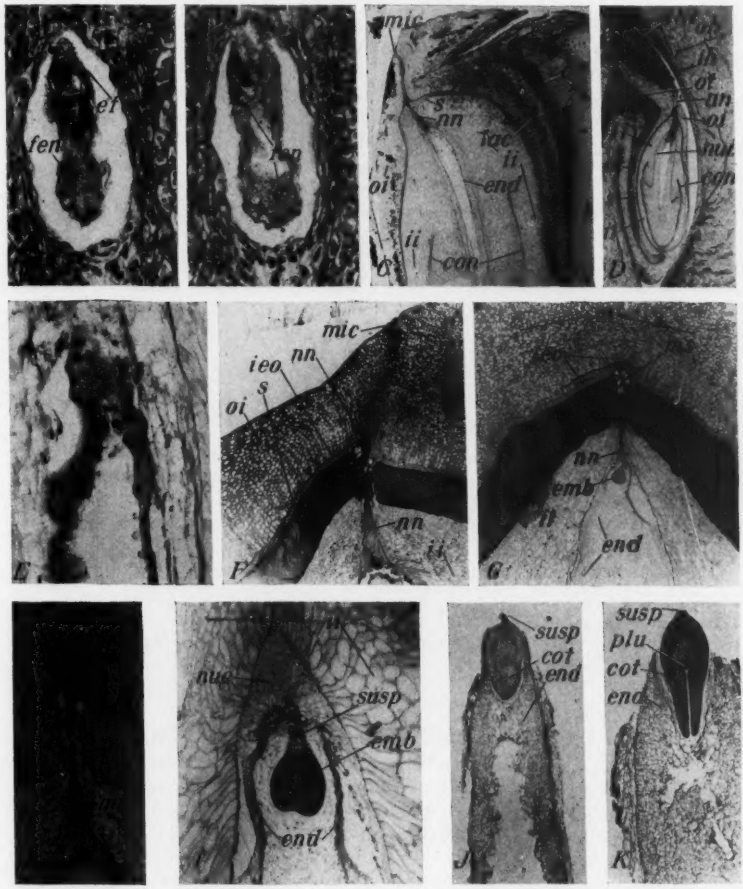

FIGURE 3.—*A*, *B*, Successive sections of embryo sac, showing four free endosperm nuclei and fertilized egg; April 10, 1941. *C*, Longitudinal section of micropylar half of developing ovule showing ovular trace, micropylar opening through outer integument and developing shell, extension of nucellar neck through micropylar opening, remains of nucellus lining embryo sac, and ends of conducting system extension of ovular trace embedded in inner integument tissue; May 22, 1940. *D*, Longitudinal median radial section of ovule, showing vascular supply to ovule and the connection of nucellar neck to obturator through micropyle; April 19, 1941. *E*, Longitudinal section through micropylar end of embryo sac showing simultaneous divisions of free endosperm nuclei; June 6, 1941. *F*, Longitudinal median radial section through micropylar end of seed, showing formation of shell from outer epidermis of inner integument, development of inner epidermis of outer integument as lining of the upper part of micropylar canal, and club-shaped cells of shell forming micropylar aperture through which nucellar neck extends; July 1, 1940. *G*. Longitudinal median tangential section through micropylar end of seed, showing further development of parts mentioned in *F*; note attachment of embryo to nucellar neck; June 28, 1940. *H*, Longitudinal median tangential section of micropylar opening in shell, showing extension of nucellar neck through aperture; June 21, 1940. *I*, Many-celled embryo and suspensor attached to basal position of nucellar neck; June 28, 1940. *J*, Embryo with suspensor and cotyledon primordia surrounded by endosperm cells; degenerating nucellus and inner integument cells surround the endosperm; July 18, 1940. *K*. Further development of embryo; July 16, 1940. *A* and *B*, × 180; *C* and *D*, × 10; *E*, × 80; *F* and *G*, × 15; *H*, *J*, and *K*, × 20; *I*, × 40. *con*, Conducting tissue; *cot*, cotyledon; *ef*, fertilized egg; *emb*, embryo; *end*, endosperm; *fen*, free endosperm nuclei; *ieo*, inner epidermis of outer integument; *ih*, inner hull; *ii*, inner integument; *lac*, latex cells; *mic*, micropyle; *ms*, modified cells of shell at micropylar opening; *nn*, nucellar neck; *nuc*, nucellus; *obt*, obturator; *oh*, outer hull; *oi*, outer integument; *ot*, ovular trace; *plu*, plumule primordium; *s*, shell of seed, or outer epidermis of inner integument; *susp*, suspensor.

micropylar end the two tissues are separated by a more or less persistent inner epidermis of the inner integument (fig. 3, *D*).

As endosperm tissue develops, the nucellus, with the exception of the region making up the nucellar neck, gradually degenerates and is absorbed as the embryo sac increases in size. By the time cell wall formation begins in the endosperm, usually about the middle of June, the major part of the nucellus has been absorbed. The tissue of the inner integument, however, is more persistent, and, though the cells adjacent to its inner epidermis show evidence of collapse and distortion, the tissue is intact until the centrifugal growth of the endosperm crushes the remaining cells against the lignified shell. In the mature seed, the crushed tissue adheres to the shell as a dry, fuzzy layer and is termed the pellicle. Thus, for the most part, the pellicle is made up of remnants of inner integument but may also contain some elements of the conducting tissue and perhaps a small portion of nucellar fragments.

MICROPYLE

The micropyle is commonly defined as the orifice, or aperture, in the seed coat through which the pollen tube enters the embryo sac. Prior to and at the time of fertilization in the tung ovule the nucellus extends through the micropyle and connects with the papillate cells of the obturator (figs. 1, *D;* 3, *D, F;* 4, *E*). The micropylar aperture penetrates the inner and outer integuments. Later, the micropylar canal extends through the shell and the aperture formed by loosely connected modified cells (fig. 3, *H*). Living parenchyma tissue of the nucellar neck may be found in the micropylar canal as late as the middle of June, at which time the cells of the shell begin to lignify (fig. 3, *H*).

CHALAZA

The ovular trace begins to differentiate in the early stages of ovule development (fig. 1, *A*, *D*) and is present and functional throughout the development of the ovule, embryo sac, and early stages of endosperm and embryo development (fig. 3, *C*, *D*). As the outer epidermis of the inner integument differentiates into the cells of the shell, an orifice is left in the chalazal end of the shell through which the ovular trace develops (fig. 3, *D*). Spiral elements characteristic of vascular tissue are found in the conducting tissue extensions of the ovular trace inside the shell.

GROWTH OF SHELL

The shell of the tung seed differentiates from the outer epidermis of the inner integument. During the early development of the ovule after fertilization the shell appears to be made up of both the outer epidermis of the inner integument and the inner epidermis of the outer integument. Both layers of cells are made up of isodiametric cells with somewhat thickened cell walls (fig. 4, *A*). The inner layer, i. e., the outer epidermis of the inner integument, begins to differentiate into shell during the latter part of May (fig. 4, *C*). The cells of the micropylar region are the first to show signs of elongation. Differentiation of the remaining cells takes place rapidly. The shell attains its maximum thickness in about 1 month and the cell walls

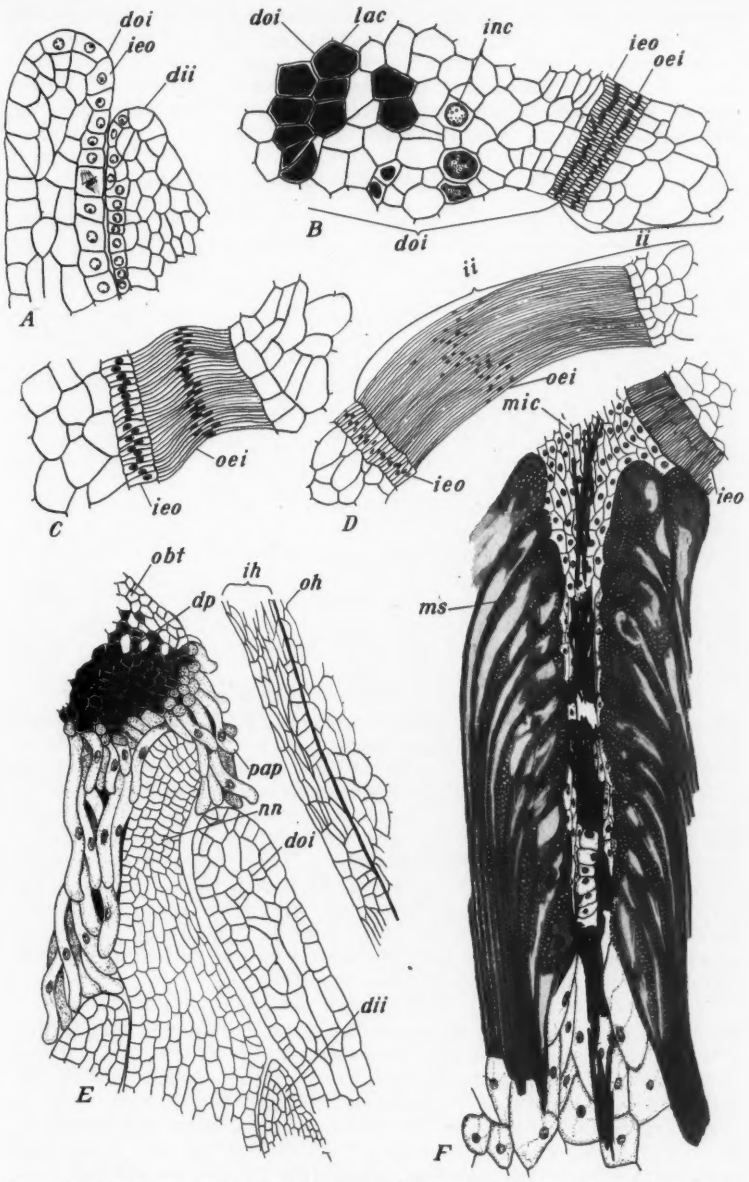

FIGURE 4.—*A–D*, Development of shell from outer epidermis of inner integument. *A*, March 28, 1941; ×385. *B*, April 25, 1941; × 170. *C*, May 22, 1941; × 170. *D*, July 15, 1941; ×90. *E*, Papillate cells of obturator in contact with nucellar neck; × 175. *F*, Micropylar opening with modified cells of shell, showing nuclei and pitted walls; × 125. Drawings made with the aid of the camera lucida. *dii*, Dorsal inner integument; *doi*, dorsal outer integument; *dp*, cells containing dense protoplasm and inclusions; *ieo*, inner epidermis of outer integument; *ih*, inner hull; *ii*, inner integument; *inc*, inclusions; *lac*, latex cells; *mic*, micropyle; *ms*, modified cells of shell at micropylar opening; *nn*, nucellar neck; *obt*, obturator; *oei*, outer epidermis of inner integument; *oh*, outer hull; *pap*, papillate cells of obturator.

immediately begin to lignify. Sections made as late as July 15 show these cell walls to be perforated with pits (fig. 4, *F*). The cells of the inner epidermis of the outer integument becomes more elongated and more closely compacted and have thicker walls than the bordering parenchyma, but they remain about one-tenth to one-fifteenth the size of the cells of the shell (fig. 4, *F*).

DISCUSSION OF OBSERVATIONAL DATA

Embryo sac formation in the tung ovule is apparently of the eight-nucleate normal type, and there are no outstanding deviations from the normal. However, there are certain accessory structures and tissues that warrant some discussion. Probably the outstanding deviation from the normal anatropous type of ovule development is the presence of a nucellar neck that extends through the micropyle. This structure is first visible at the time of the initiation of the first ovular primordium and persists until the seed is almost ripe. The neck extends beyond the ends of the integument primordia into the locular cavity and eventually comes in contact with papillate cells of the obturator immediately at the micropylar end of the anatropous ovule. Since the tissue of the obturator and its papillate extensions consist of cells having dense protoplasmic contents with great affinity for biological dyes, it is suggested that the nucellar neck and papillate cells form a bridge for the translocation of nutritive materials from the tissue of the obturator to the embryo sac. At the time of embryo sac formation and throughout the growth of the embryo, the nucellar neck is in direct contact with the suspensor and the dark-staining cells at the obturator end of the bridge are in proximity to the vascular complex in the funicular region of the placenta.

The developing ovule is also in direct contact with the main vascular system by means of an ovular trace that is recognizable from the time the first primordia of the ovule appear. At the time of fertilization the ovular trace is clearly defined as a region of elongated dark-staining cells extending the length of the anatropous ovule from the funiculus through the center of the ventral outer integument to the chalazal end. The trace turns upward abruptly at the base of the ovule and penetrates through the outer epidermis of the inner integument, where it connects with a bell-shaped system of elongated cells, which exhibit spiral elements and without doubt serve to extend the ovular trace into the ovule about three-fourths the length of the ovule. Thus the nucellus is in direct contact with the main vascular system of the axis throughout the development of the ovule and seed.

Therefore, the growing embryo may receive nourishment in any one or all of three ways: (1) It is in direct contact with a nucellar neck, which is itself rich in aleurone grains and is indirectly connected to the main vascular complex through the rich storage tissue of the obturator; (2) it may receive nourishment from the cellular contents of the degenerating nucellus encroached upon by the enlarging embryo sac; (3) it may receive nourishment from the extension of the ovular trace during and after nucellar resorption. It is also possible that the inner parenchymatous layers of the inner integuments contribute to the nutrition of the contents of the embryo sac, though it is more likely that this tissue, together with the outer integument, has some

part in the tremendous elongation of the outer epidermal cells of the inner integument, which form the shell of the seed.

There is no definite tapetum inside the shell, unless the inner epidermal cells of the inner integument immediately adjacent to the nucellar neck may be so designated. There is no thickening of the cell walls of these epidermal cells such as is usually reported for tapetal development.

The micropylar foramen lies between the tips of the outer integument and penetrates the shell by means of a small orifice formed by enlarged, pyriform, pitted cells of the shell. The foramen encloses nucellar neck tissue from the beginning of ovule formation throughout the development of the seed. Toward the latter part of seed development the parenchymatous neck tissue is crushed, so that only remnants of dark-staining material remain in the micropylar canal.

The inner epidermis of the outer integument completely surrounds the outer circumference of the shell. The cells of this layer are heavy-walled and somewhat elongated. In the region bordering the upper part of the micropyle, there is some further differentiation of the cells into what appears to be a protective layer. There is an opening in the layer at the tip of the chalazal end opposite that in the shell through which the ovular trace penetrates.

The structure and position of the pitted cells of the shell indicate the possible methods by which the developing embryo and endosperm may receive supplementary nourishment from outside the shell. It is to be noted that the neck of the nucellus extends through the micropylar end of the shell and is in contact with the obturator, while the conducting tissue of the ovular trace penetrates the chalazal end of the shell. Also, the pitted nature of the cell walls of the shell as a whole may permit some translocation or interchange of water and nutrients.

MORPHOLOGY AND PROBABLE FUNCTIONS OF THE OVULE AND ACCESSORY TISSUES

OUTER EPIDERMIS OF OUTER INTEGUMENT

The layer of cells forming the outer epidermis of the outer integument completely surrounds the anatropous ovule with the exception of the region of attachment at the funiculus and the micropylar opening. The cells are cubical or slightly rectangular, and the outer surface is eventually coated with cuticle. The main function of this layer is protection of the underlying parenchyma.

PARENCHYMA OF OUTER INTEGUMENT

The parenchyma of the outer integument is 40 to 60 cells thick by the time maximum size of the ovule is attained. The cells are irregular polygons with comparatively thin walls. The tissue is interspersed with patches of dark-staining cells with rich protoplasmic or latex content. Various types of crystals and other inclusions are found in the cells.

INNER EPIDERMIS OF OUTER INTEGUMENT

When the ovule has attained maximum size, the cells of the inner epidermis of the outer integument are elongated radially and have

comparatively thick walls. The layer is evidently quite firmly attached to the adjacent cells composing the shell and formed by the outer epidermis of the inner integument and completely surrounds them except in two regions; it forms a protective border for the micropylar canal between the apical portion of the outer integument, but it does not cover the micropylar opening; it has an opening in the chalazal tip of the ovule that permits the ovular trace to penetrate.

OUTER EPIDERMIS OF INNER INTEGUMENT

The layer forming the outer epidermis of the inner integument is the hard bony shell of the seed. There is great radial elongation of these epidermal cells to form the sclerified, pitted elements that make up the shell. The cells may be straight, slightly bowed, or knee-shaped. There are two openings in the shell. At one end of the seed claviform cells form the lower portion of the micropylar canal. At the chalazal end an opening permits the penetration of the ovular trace. The cells bordering the chalazal canal are shorter but otherwise similar in shape to the remainder of the cells forming the shell.

According to Netolitzky (*10*), the development of the outer epidermis of the inner integument into modified cells is characteristic of entire families, such as the Malvaceae, Tiliaceae, Bombacaceae, and Sterculiaceae, and is also found in the Euphorbiaceae, Dipterocarpaceae, Cistaceae, Bixaceae, Violaceae, Passifloraceae, and Thymelaeaceae. Pammel (*11*) also discussed the coils of the shell found in the euphorbiaceous seeds.

PARENCHYMA OF INNER INTEGUMENT

In the early stages of endosperm and embryo development the parenchyma of the inner integument makes up about 60 to 75 percent of the volume of the seed inside the shell. As the shell, endosperm, and embryo increase in size, the parenchyma is crushed and is almost, if not entirely, absorbed.

NUCELLUS

The nucellus may be divided into two regions with regard to probable function: (1) An upper or micropylar region above the embryo, which serves as a nucellar neck structure, extending from the suspensor of the embryo, through the micropylar canal, to the papillate cells of the obturator; and (2) a lower or chalazal region which is encroached upon and finally absorbed by the enlarging embryo sac. In both parts of the nucellus the cells contain rich protoplasm. The nucellar neck region, however, retains its rich protoplasm much longer and, furthermore, is especially rich in aleurone grains.

CONDUCTING TISSUE

The ovular trace penetrates the seed coat through the chalazal opening and branches to form a bell-shaped conducting system made up of elongated dark-staining cells that lie close to the shell but do not touch it. A thin layer of inner integument parenchyma lies between the conducting system and the shell until it is crushed by the

centrifugal expansion of the endosperm. The conducting system extends upward about two-thirds to three-fourths the length of the ovule, where it meets the inner epidermis of the inner integument. Thus a boundary is established between the nucellus and the parenchyma of the inner integument throughout the entire length of the ovule. Eventually, spiral elements develop in the conducting tissue, the parenchyma of the inner integument is partly or completely absorbed, the conducting elements come to lie adjacent to the shell and probably go to make up part of the membranelike pellicle found on the inner surface of the mature shell.

ENDOSPERM

The endosperm nuclei begin to increase in number very soon after triple fusion has occurred. At first, the free endosperm nuclei orient themselves in a single layer along the periphery of the embryo sac. This layer enlarges with the lengthening embryo sac until about the middle of June, when rapid cell division occurs, first in the region surrounding the embryo and later along the whole peripheral layer of endosperm. Cell wall formation follows almost immediately, and a solid web of tissue soon lines the entire embryo sac. The center of the embryo sac remains open, permitting extension in length of the cotyledons of the embryo.

EMBRYO

Although fertilization of the egg cell takes place within 36 hours after pollination, subsequent embryo growth is comparatively slow. The embryo attains about the 16- to 32-cell stage usually by the middle of June, but remains microscopic in size until about July 1. This delayed growth of the embryo may be caused by a lack of available nutritive materials which, up to about July 1, are required for growth of structural elements of the fruit.

The ovule attains its maximum size about July 1. The shell, developing from the outer epidermis of the inner integument by rapid radial elongation of the epidermal cells, begins to lignify at about the same time that the endosperm and embryo begin the rapid growth characteristic of these structures throughout July to late August. By this time, the internal structural development of the ovule is completed and the various parts have attained full size. The ovule parts are oriented in the manner of mature seeds. There is no further growth by cell division or enlargement.

The mature fruit consists of from one to many, usually five, seeds enclosed by a subwoody pericarp. The shell of the seed is covered with a reddish-brown dust, the remains of outer integument tissue. The seeds are somewhat flattened on the dorsal side and are slightly bulged at the ventral side of the micropylar end. The chalazal end of the seed is slightly smaller than the micropylar end. The seed coat is rough and bony and forms a point directly over the micropyle and is slightly indented at the chalazal end. The seed is oriented in the fruit so as to be parallel with the central placenta, and the micropylar end of the seed is directed toward the apex of the fruit and the chalaza toward the stem end. The kernel consists chiefly of en-

dosperm in which the embryo is embedded. The radicle of the embryo lies just below the micropylar tip formed by the seed coat, and the two flat cotyledons are oriented in the center of the seed so as to parallel with the wide axis of the seed and at right angles to the placenta.

The mature seed, when dry, is seldom filled completely. A space is usually found between the cotyledons extending in length from the plumule primordia to the ends of the cotyledons and in width from margin to margin of the cotyledons. The size of the space depends upon the amount of endosperm filling the seed.

SUMMARY

The primordia of the tung ovule develop in a manner typical of the normal anatropous ovule, with the exception of the nucellus, which protrudes through and beyond the micropylar opening formed by the two integuments.

The megaspore mother cell is hypodermal in origin. Two transverse divisions produce a row of four megaspores, of which the outer three degenerate. The chalazal megaspore enlarges and divides to form two nuclei, one orienting itself in the micropylar end of the embryo sac and the other in the chalazal end. These two nuclei then divide, thus producing two micropylar and two chalazal nuclei. The four nuclei then undergo a final division, producing four nuclei at each end of the sac. One nucleus from each group then migrates to the center of the sac, thus forming in the conventional manner a normal eight-nucleated embryo sac, consisting of an egg and two synergids in the micropylar end, three antipodals in the chalazal end, and two polar nuclei at or near the center of the sac.

The egg nucleus is fertilized between 24 and 36 hours after pollination. Synergids degenerate early, sometimes before fertilization. Antipodals also degenerate at the time of fertilization or soon after.

The fertilized egg does not immediately begin to divide but rests for a period of 1 to 2 weeks.

Embryos collected 2 to 4 weeks after pollination were found in the 8-cell stage. At 6, 7½, and 10 weeks after pollination, most embryos were in the 32-cell stage. There is a short rest period at the 32-cell stage during the latter part of May or early June. Further divisions may occur throughout June, but rapid cell division does not begin until after July 1, when cell division and consequent increase in embryo size are rapidly accelerated. Full size is usually attained late in August.

Endosperm development was observed to begin with the first division of the $3n$ fusion nucleus in material collected 52 hours after pollination. Subsequent divisions occur regularly but relatively slowly, forming a single layer of free endosperm nuclei around the periphery of the embryo sac until about mid-June, when simultaneous cell divisions begin and cell walls are formed. Rapid endosperm development is concurrent with rapid embryo growth, and the seed is usually filled with endosperm by late August.

Morphological details of the formation of the shell, micropyle, pellicle, and chalaza are discussed in connection with their respective roles in the physiological functions of the ovule and accessory tissues.

LITERATURE CITED

(1) ABBOTT, C. E.
1929. FRUIT-BUD DEVELOPMENT IN THE TUNG-OIL TREE. Jour. Agr. Res. 38: 679–695, illus.

(2) D'AMATO, F.
1939. RECERCHE EMBRIOLOGICHE E CARYOLOGICHE SUL GENERE "EUPHORBIA." Nuovo Gior. Bot. Ital. 46: 470–509, illus.

(3) DICKEY, R. D., and REUTHER, W.
1940. FLOWERING, FRUITING, YIELD AND GROWTH HABITS OF TUNG TREES. Fla. Agr. Expt. Sta. Bul. 343, 28 pp., illus.

(4) LYON, F. M.
1898. A CONTRIBUTION TO THE LIFE HISTORY OF EUPHORBIA COROLLATA. Bot. Gaz. 25: 418–426, illus.

(5) MCCANN, L. P.
1942. DEVELOPMENT OF THE PISTILLATE FLOWER AND STRUCTURE OF THE FRUIT OF TUNG (ALEURITES FORDII). Jour. Agr. Res. 65: 361–378, illus.

(6) ——— COOK, W. S., and CAMPBELL, C. R.
1941. FACTORS AFFECTING TIME OF INITIATION AND RATE OF DEVELOPMENT OF PISTILLATE FLOWERS OF THE TUNG TREE. Amer. Soc. Hort. Sci. Proc. (1941) 39: 157–160, illus.

(7) MODILEWSKI, J.
1910. WEITERE BEITRÄGE ZUR EMBRYOBILDUNG EINIGER EUPHORBIACEEN. Deut. Bot. Gesell. Ber. 28: 413–418, illus.

(8) ———
1910. ZUR EMBRYOBILDUNG VON EUPHORBIA PROCERA. Deut. Bot. Gesell. Ber. 27: 21–26, illus.

(9) ———
1911. ÜEBER DIE ANOMALE EMBRYOSACKENTWICKLUNG BEI EUPHORBIA PALUSTRIS L. UND ANDEREN EUPHORBIACEEN. Deut. Bot. Gesell. Ber. 29: 430–436, illus.

(10) NETOLITZKY, F.
1926. ANATOMIE DER ANGIOSPERMEN-SAMEN. *In* Linsbauer, K., Handbuch der Pflanzenanatomie. Lfg. 14, Bd. 10, illus. Berlin.

(11) PAMMEL, L. H.
1892. ON THE SEED-COATS OF THE GENUS EUPHORBIA. Acad. Sci. St. Louis, Trans. 5: 543–568, illus.

(12) SCHLOTTERBECK, J. O.
1898. THE DEVELOPMENT OF THE SEED OF MELAMPYRUM PRATENSE AND OF CROTON TIGLIUM. Amer. Assoc. Adv. Sci. Proc. (1897) 46: 275–278.

(13) SCHNARF, K.
1927–29. EMBRYOLOGIE DER ANGIOSPERMEN. *In* Linsbauer, K., Handbuch der Pflanzenanatomie. Lfg. 23, Bd. 10/2, illus. Berlin.

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